Right ventricular assessment using ultrasound in a healthy, multi-ethnic population

Willis, James

Award date:
2014

Awarding institution:
University of Bath

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James Alexander Willis

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Pharmacy & Pharmacology

April 2014

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Acknowledgements

Firstly, my thanks must go to my wife Katie for supporting me through this endeavour. Katie has remained patient and understanding and provided unwavering encouragement from start to finish. I must also thank my parents for their encouragement and enthusiasm.

Both Dr Jacob Easaw (JE) and Professor Rex Tyrrell have provided support guidance and advice as my supervisors, for which I am grateful. Thanks must also go to Helen Twemlow and the staff of the Cardiac Centre at the Royal United Hospital for allowing me to pursue this challenge.

Individual thanks include both Joe Sparey (JS) and Amy Szewiel (AS) for assisting me with the scans. Dr Rajesh Shah for his logistical planning and valuable feedback. Dr Daniel Augustine (DA) and Dr Daniel Knight for providing constructive advice, logistical planning and a sympathetic ear, Dr David Oxborough for his time to discuss the virtues of scaling and Cardiac Physiologist, Fiona Burchard for reading several drafts of the thesis and always providing helpful feedback.

Finally, I am grateful to Katherine for teaching me to use my initiative.

List of abbreviations

2D –	Two dimensional
3D-	Three dimensional
A2C-	Apical two chamber
A3C-	Apical three chamber
A4C-	Apical four chamber
ANOVA-	Analysis of Variance
AOV-	Aortic Valve
ARVC-	Arrhythmogenic Right Ventricular Cardiomyopathy
ASE –	American Society of Echocardiography
BMI-	Body Mass Index
BSA-	Body Surface Area
CHF-	Congestive Heart Failure
cMRI-	Cardiac Magnetic Resonance Imaging
COV-	Coefficient of variation
CSV-	Crista Supraventricularis
CT -	Computed Tomography
CVD-	Cardiovascular Disease
CW-	Continuous Wave
EF-	Ejection fraction
GDSRe-	Global Diastolic Early Strain Rate
GDSRa-	Global Diastolic Late Strain Rate
GPSS-	Global Peak Systolic Strain
GPSSR-	Global Peak Systolic Strain Rate
ICC-	Intraclass correlation coefficient
IVSd-	Interventricular septum diastole
LOA-	Limits of agreement
LRV-	Lower reference value
LV –	Left Ventricle
LVIDd-	LV internal dimension diastole
LVIDs-	LV internal dimension systole
LVOT-	Left Ventricular Outflow Tract
MESA –	Multi Ethnic Study of Atherosclerosis

MRI-	Magnetic Resonance Imaging
MV-	Mitral Valve
ns-	Non significant
PAH-	Pulmonary Arterial Hypertension
PH-	Pulmonary Hypertension
PLAX-	Parasternal Long Axis
PSAX-	Parasternal Short Axis
PSS-	Peak Systolic Strain
PSSR-	Peak Systolic Strain Rate
PV-	Pulmonary Valve
PVC-	Pulmonary Valve Closure
PW-	Pulsed Wave
PWd-	Posterior wall diastole
R^2-	Adjusted regression coefficient
r-	Correlation coefficient.
RA-	Right Atrium
ROI-	Region of interest
RV –	Right Ventricle
RVD -	Right Ventricular Dimension
RVEDA-	Right ventricular end-diastolic area.
RVESA-	Right ventricular end-systolic area.
RVFAC-	Right ventricular fractional area of change
RVOT-	Right ventricular outflow tract
STE –	Speckle Tracking Echocardiography
TDI-	Tissue Doppler Imaging
TSC	Tanners Special Circumstance
TSD-	Tukeys Honestly significant difference test
TTE-	Transthoracic Echocardiogram
TV-	Tricuspid Valve
URV-	Upper reference value

Abstract

Current published reference values for right ventricular (RV) echocardiographic assessment do not adequately account for the reported changes in size and function that can occur due to gender, age and body size. In addition, the potential effects on RV dimensions of ethnic variation within healthy volunteers are relatively unknown. The aim of this study was to better understand the potential differences in RV size that may result from gender, body size, ethnicity or age in a healthy volunteer group.

255 healthy volunteers (mean age 41+11 years 53% male) from five ethnic groups (Indian, Chinese, Malay, European, Afro-Caribbean) were recruited. Assessment of RV size was conducted using 2D calliper measurements, 3D volume, ejection fraction (EF) and RV deformation characteristics (peak systolic strain (PSS) and strain rate (PSSR)). Intraobserver, interobserver and test-retest scenarios were performed to assess measurement variability and reliability.

Regression analysis identified gender, age, body size and ethnicity as significant coefficients of 2D RV size and 3D volume. European and Afro-Caribbean 2D and 3D measurements were on average larger than the remaining ethnic groups whilst males exceeded females. Allometric scaling of the raw 2D data to body surface area (BSA) resulted in gender and body size independent measurements whilst measurements between ethnic groups displayed varying significance on both RV size and volume. Increasing age resulted in a number of significant interactions with size and volume.

Gender differences were apparent for both 3D ejection fraction (EF) and strain measurements. Females displayed both higher 3D EF and PSS using an RV freewall approach. The reproducibility of the RV measurements varied depending on the method used and the scenario undertaken.

Gender, age and body size are important determinants of 2D linear deformation, and 3D acquired RV measurements. These results highlight the need for

appropriate allometric scaling of RV measurements to BSA, whilst highlighting the importance of documenting ethnicity.

1. General Introduction

1.1. Linear Dimensions

Transthoracic echocardiography, despite its development into multiple sub-disciplines, remains one of the pillars of cardiac assessment (Macdonald et al., 2008). Quantitative evaluation of chamber size and function is still one of the most challenging yet clinically requested investigations (Lang et al., 2005); the result can have significant clinical bearing on both immediate and future management of patients and it is therefore imperative that a standardised methodology is devised for both assessment and reporting. It is for this reason that a publication including guidelines on the assessment of cardiac structure and function remains one of the most widely cited in the field (Lang et al., 2005).

These guidelines tend to focus on the assessment of 'normal' or 'healthy' populations often constructed in a meta-analysis style. By constructing reference ranges using data from a normal population, deviations outside this can be reported accordingly, resulting in a confirmation of, or change to, clinical management. Research and the creation of normative left ventricular (LV) values has been essential for the interpretation of and comparison between normal and abnormal cardiac function, demonstrating the clinical importance of features such as reduced systolic function. There is, however, less research into the assessment and measurement of right ventricular (RV) size and function compared to studies involving LV assessment (Kawut et al., 2011).

Compared to the systemic circulation, the physiology and anatomy of the RV is centred around lower pressures and workload, resulting from differences in morphology and perfusion due to an increased vascular bed (Kawut et al., 2011). The contribution made by the RV musculature is designed to ensure the maintenance of adequate pulmonary pressure under various loading conditions, which ensures good passage of deoxygenated blood to the gas exchange membranes of the lungs. In addition this low pressure system prevents tissue and organ congestion (Bleeker et al., 2006) and as a result, requires less myocardium than the more muscular LV.

Historically the RV was seen as a mere conduit for the blood to be moved through the lungs, with no additional purpose but to act as a versatile container. Research within the last 20 years, however has shown that the RV adapts to a number of physiological conditions such as endurance exercise (La Gerche et al., 2008) and pathological conditions such as congenital heart disease and pulmonary hypertension (PH) with the resulting RV dysfunction leading to considerable morbidity and mortality (Davlouros et al., 2006)

In addition to congenital pathology, right sided heart failure can also be caused by cardiomyopathies, coronary artery disease (both directly and indirectly resulting from LV involvement), acute and chronic pulmonary embolism and valvular heart disease (Badano, 2011). RV function and performance often predicts outcomes for patients with cardiovascular or pulmonary disease. In order to assess and monitor the RV reliably, an in-depth understanding of the process of right heart assessment using echocardiography is essential (Davlouros et al., 2006, Mehta et al., 2001, Bleeker et al., 2006). Furthermore, the interaction between a dysfunctional RV and LV is interlinked by ventricular interdependence. This underpins the need for detailed assessment of both cardiac chambers (Bleeker et al., 2006).

Whilst echocardiographical knowledge about the LV has improved with indices of normality for size, function, volume and deformation, the knowledge base surrounding the RV has lagged. Despite this, echocardiography still remains the first line imaging technique to screen for RV impairment (Davlouros et al., 2006) (Badano, 2011).

During the conception of this project, the current recommendations for chamber quantification were described in a joint report from:
American Society of Echocardiography's Guideline and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. European Society of Cardiology (ESC) (Lang et al., 2005)

This report represented a significant contribution, in both methodology and quantification, from two of the largest societies in the world. Nonetheless the focus of this paper remained predominately on assessment of the LV with some detail pertaining to the RV. Despite acknowledging in the text and prescribed guidelines, differences in LV chamber size for men and women, and the potential for simple linear scaling of results to BSA, there remained a reduced array of tools dedicated to the quantification of RV size and function. In 2010 the American Society of Echocardiography (ASE) published guidelines for the assessment of the RV, based on meta-analysis data. This will be discussed later in the chapter, alongside other key papers in the chronology of RV assessment.

In spite of this disparity in chamber assessment, there is compelling evidence that differences in both the structure, and function of the heart can be found not only between men and women, but also different ethnic groups, and with increasing age (Willis et al., 2012, Fernandes et al., 2011, Kawut et al., 2011, Maceira et al., 2006b, Tamborini et al., 2010, Maffessanti et al., 2013). In the face of advances in imaging techniques, such as cardiac magnetic resonance imaging (cMRI) and computed tomography (CT), echocardiography remains one of the most commonly used, non-invasive, imaging techniques available and thus these well recognised changes should be accounted for when utilising this technique.

There is a growing body of evidence surrounding the echocardiographic assessment of conditions such as PH or Arrhythmogenic right ventricular cardiomyopathy (ARVC). This utilises both traditional and new techniques which have embraced developments in imaging technology. In spite of the renewed interest in the structure, size and function of the RV, there are currently only limited normative studies that provide up-to-date and comprehensive assessments of not only the technique but also outcomes in a robust population sample. Major criteria for the confirmation of ARVC include (amongst other tests) RVOT-1 measurements >36mm and RVOT-3 measurements >32mm (Marcus et al., 2010). These guidelines also include both minor criteria and measurements indexed to BSA to account for the often large BSA readings derived from certain groups of athletes.

Despite the focus of research concerning the LV, the effect of differences in gender, ethnicity, and with age on the equally important RV are less well understood. The knowledge base surrounding echocardiographic differences in healthy groups of differing ethnicity is limited. It is limited to both the standard two-dimensional (2D) level, using recognised methodology, and at a more advanced level with both myocardial deformation and volume analysis.

Given the fact that there is less research into both normal and abnormal RV function, there is a clear need to ensure that the echocardiographic indices of normality for the RV are relevant, robust and up-to-date. In spite of this, prominent articles from some 30 years ago still remain as regular reference (Foale et al., 1986).

1.2. Speckle Tracking Echocardiography.

New methods of cardiac assessment such as speckle tracking echocardiography (STE) have been studied in a number of varying pathological conditions often compared to a small matched control group (Teske et al., 2009b, Fukuda et al., 2011). Whilst it is imperative to advance the understanding of how disease states such as PH and ARVC changes the myocardial interaction, we must also appreciate that these techniques come with limitations. It is only with larger studies examining the normal population that we can appreciate the often subtle differences that can occur with disease.

STE within a healthy population has been reported in larger epidemiological studies where the focus has been on the LV (Dalen et al., 2010). One such study conducted in 2006 by a Danish group, The Nord-Trøndelag health study (The HUNT study), described the benefits and technical limitations of STE within their normal population study (Dalen et al., 2010).

The same group also delivered a second paper based on their experience conducting the study, detailing the interobserver reproducibility of several STE based techniques (Thorstensen et al., 2010). The same level of detail in the use of

STE is yet to be fully described for the RV, however, whether in terms of its positive attributes or its possible limitations as a technique.

1.3. Three dimensional imaging

3D volumetric analysis has made rapid advances, even within the timeframe of this thesis. Newer analysis software now exists which is designed to remove some degree of human bias; as well as systems that are more integrated, allowing better imaging at the point of interest, rather than post-acquisition analysis. Despite these improvements in the technology available, traditional RV assessment remains hampered both because of lack of access to dedicated RV analysis software and imaging capabilities, and because of the complex anatomy and reduced endocardial definition, secondary to imaging difficulties, which limit assessment of the RV. This remains a challenge within this field that must be acknowledged and accounted for when making clinical assessments.

A better understanding of both the techniques currently available, and the normal reference ranges for each, will allow for increased knowledge development surrounding RV assessment in various pathological conditions.

This investigation into the normal RV represents one of the most robust studies to date, both in terms of sample populations and methodology. By clarifying and establishing normal criteria within this group of healthy volunteers, it is hoped that future work will be able to detect ethnic, gender or age associated differences across various imaging modalities.

1.4. Study Aims and Hypothesis

This study has been designed to bring together several methods of echocardiographic assessment and present these techniques and their limitations whilst assessing the right heart. These techniques will assess in detail the size and function of the RV within a diverse, multi-ethnic group of healthy volunteers. The inclusion of ethnicity as a variable within the study design will allow for its assessment as a potential influence on RV size and function alongside recognised influential parameters such as body size, age and gender. A primary hypothesis to be tested in this thesis is therefore that ethnicity will influence RV measurements. Several sub hypotheses will also be tested, namely that: 1) When accounting for body size, gender differences in 2D RV measurements are no longer significant. 2) When accounting for body size, gender differences in 3D RV volumes are no longer significant. 3) Increasing age will lead to changes in both RV size and volume and 4) Allometric scaling is more effective at producing body size independent RV measurements compared to ratio-metric scaling.

In order to test these hypotheses it will be necessary to establish normal 2D, 3D and deformation characteristics for RV echocardiographic parameters in a large heterogeneous population. In addition it is essential to establish the effect body size can have on RV measurements, determine the extent of any gender differences, ascertain how age may effect these measurements and establish the variability, reliability and agreement of these measurements between users.

The discussion of the more common variables and their influence on RV dimensions will be further assessed with the use of ratio-metric and allometric scaling. By utilising these linear and non-linear techniques to remove variables such as body size from predicted RV dimensions, it is anticipated that this may further help understand how influential ethnicity is on body size independent RV measurements and the determination of reference values.

Equally, functional assessment of the RV remains imperative and in addition to RV size, this study will investigate the effects of ethnicity, age, gender and body

size on measures for RV deformation, calculated using STE, and RV volumes derived from 3D acquisitions. A detailed examination of the echocardiographic assessment is discussed in later sections of the literature review to place in context the comparison to other methods, and in addition, how more modern technologies provide alternative assessment methods. These techniques will be further scrutinised regarding their measurement variability, reliability and subsequent agreement between users and acquisition.

The thesis provides an extensive assessment of the RV that will provide reference ranges for many of the common techniques used to assess the right heart, from a diverse healthy population. The issues surrounding this ethnic diversity however are complex and challenging.

To ensure that the study demographic was truly diverse, a multi-centre approach was utilised, which involved recruitment from centres in both the United Kingdom and Pennang, Malaysia. To make certain that the results could be applied in a logical manner should it be required, recruitment was then focused on five ethnic groups: Indian, Chinese, Malay, European and Afro-Caribbean. These five groups were identified because of their relative size as a demographic within the three recruitment centres and census data.

Details regarding volunteer selection are discussed later within both the literature review and methodology, where the method and limitations of other studies are discussed. It is important to recognize that the assessment of ethnicity is a complicated and sometimes controversial subject. Within the scope of this project, a broad representation of both sample size and ethnicities was not possible and therefore any recommended guidelines for size and function must also be interpreted within the clinical context of the patient.

1.5. Defining Ethnicity

Ethnicity in the context of health can be defined as:
"a group that people belong to because of shared characteristics, including ancestral and geographical origins, cultural traditions and languages" (Bhopal et al., 1997).

European and American research that focuses either exclusively on or features as a variable, subjects ethnicity and health, has in the past been considered to use poorly defined labels to describe study populations (Bhopal and Donaldson, 1998). The search for an accurate definition remains controversial for both scientific and social reasons. The definition of an ethnic group for health research that could in turn determine the care and treatment given to an individual patient has implications when patients do not fit into a particular ethnic group.

(Kaplan and Bennett, 2003) suggested that three challenges exist when reporting about race and ethnicity:
To account for the limitations of racial/ethnic data,
To distinguish between Race/Ethnicity as risk factor or risk marker,
To avoid contributing to the Racial/Ethnic division of society.

Although these challenges are very real and attempts should be made to account, distinguish and avoid the suggested pitfalls, it is also important to note that race and ethnicity do not describe the same thing and should not be used interchangeably.

(Bhopal and Donaldson, 1998) suggested that the continued use of 'race' in favour of 'ethnicity' causes the reader to interpret results as biologically constituted and scientifically recognised where as both race and ethnicity are suggested to be socially constructed. Ethnicity is regarded as reflecting more accurately, changes due to a social construction (Bradby, 2003).

Medical research within the area of ethnicity has been criticised in the past, even to the extremes of racism (Osborne and Feit, 1992). Ethnic categories used in

research are widely agreed to be unsatisfactory in both theoretical thinking and in practical terms (Bhopal, 1997). The use of fixed-response schemes to define ones ethnicity provides a simple and measureable means of identifying and categorizing groups. However it is argued that this can lead to a racialised view of human diversity (Bradby, 2003).

Methods that require a simple "tick in the box" approach to ascertain which group the subject falls into simply does not account for the worldwide variation that exists and which people use to define their own identity. An alternative approach adopted by researchers within the London Life Sciences Prospective Population (LOLIPOP) study required both maternal and paternal grandparents to originate from the Indian subcontinent in order to be defined as Indian. A similar approach for European participants was implemented, with both grandparents originating from northern Europe (Chahal et al., 2010).

By allowing subjects to describe their own ethnicity via interview or to write it in a free text manner, it has been suggested that a more accurate spread of ethnicity can be achieved incorporating people's self-identification and mitigating the excesses of fixed-response categorisation (Bradby, 2003). Although this approach may lead to increased numbers of ethnic groups being expressed by recruited volunteers, it ensures that the volunteers are given a choice.

However, the current practice within the National Health Service (NHS) is to ask participants to provide a tick box response, with the option to add free text within in the 'other' box. Therefore, to ensure that the analysis, interpretation, and correct sample size are met, practical reasons dictate that there must remain some potential to define volunteers by a group. It is recognised that this must involve their participation in arriving at this decision, resulting in the need to discuss with volunteers their perception of ethnicity and also their family heritage.

The division of ethnicity into the five groups (European, Malay, Chinese, Afro-Caribbean and Indian) does not represent an exhaustive list. Additional factors to consider are non-genetic issues such as the socioeconomic status of volunteers

alongside the number of years spent as a resident in certain countries, while, to a lesser extent, people may define their ethnicity based solely upon their religion.

1.6. Ethnicity and the Heart: Echocardiographic Evidence

Differences in the baseline physiology of healthy people from different ethnic groups are well established; with independent schools of population research such as the London Life Sciences Prospective Population (LOLIPOP) dedicated to the long-term assessment within this field (Chahal et al., 2010). Specifically, cardiac research into ethnic based differences has largely focused on the assessment of the LV, the LV in the presence of hypertension and RV studies with athletes. This is pertinent given that the incidence of hypertension amongst South Asians, and people of Caribbean and West African ancestry has been found to be raised by two- to threefold compared to the incidence in the native population in the UK alone (Cappuccio et al., 1997).

Cardiac size and function has been shown to differ between healthy members of various ethnic groups (Natori, 2006, Park et al., 2013, Fernandes et al., 2011) and in particular in the assessment of athletes (D'Andrea et al., 2012, Zaidi et al., 2013, Basavarajaiah et al., 2008, Baggish et al., 2008). These findings have been used to help distinguish pathological conditions such as hypertrophic cardiomyopathy from normal physiological adaptation to exercise (Chandra et al., 2012, Zaidi et al., 2013).

Studies have found that the extent to which these differences occur varies depending not only on ethnicity, but also on the type of sport undertaken (Rawlins et al., 2010). Importantly, recent research has also highlighted the effect of both 2D and 3D LV mass calculations on reporting ethnic differences, with a suggested over-estimation caused by 2-D geometric assumptions (Park et al., 2013).

In a recent study into the adaptation of the RV in elite athletes, regression results of RV dimension, found that within the group of elite athletes, a predictor of

increased right ventricular outflow tract (RVOT)-1 dimension was a Caucasian ethnicity (Zaidi et al., 2013). They did also note that BSA remained significant, with both displaying a similar β value ($p<0.001$ respectively). Further assessment of the observed variation (R^2) value found that only a minimal proportion of variation (2.3%) was attributed to ethnicity. The suggestion from this paper was that differences in RVOT size between black and Caucasian athletes may have resulted from their athletic training despite the significant independent prediction previously mentioned (Zaidi et al., 2013).

The evidence from the normal healthy volunteers suggests that, despite RV dimensions being corrected for body size, statistically significant differences remain between ethnic groups. These differences may result from cardiac adaptation or true genetic variability. Current thinking, however, has yet to clearly identify the genetic markers pertaining to cardiac adaptation.

A 1985 study investigated the effects of exercise training on monozygotic twins, dizygotic twins, siblings of a similar age and unrelated age and gender matched controls (Adams et al., 1985). The aim of this study was to investigate influences that may control cardiac size, specifically looking at familial influences. By using monozygotic twins, the authors hypothesised that a strong link between genetics and cardiac size would be established if, after 14 weeks of exercise training, identical gains in cardiac size were to be found within this group alone, compared to the remaining participants.

The results of Adams et al.'s study demonstrated that there was a greater degree of similarity in cardiac dimensions between both sets of siblings and twins compared to age and gender matched pairs. The authors concluded that a combination of environmental and genetic factors influenced cardiac dimensions more than genetic factors alone (Adams et al., 1985). The bigger question as to whether genetics will be able to answer many of the common health concerns is still some way from being answered, with further studies, working with a specific aim to investigate the genetic make up of specific ethnic groups still required (Collins, 2004).

1.7. Multi-Ethnic Study of Atherosclerosis (MESA)

Several large, observational based studies have dominated research into cardiac based ethnic differences. The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicentre prospective cohort study. The study, initiated in 2000, initially aimed to stratify cardiovascular disease (CVD) over a seven-year period in different ethnic groups. 6,500 men and women, aged 45-84 years were recruited to this study.

The ethnic groups consisted of Whites, African Americans, Hispanics and Asian Americans (Chinese descent). Volunteers recruited to the study were asymptomatic and free from any known CVD. Baseline demographics, a CT scan and cMRI were performed in all subjects. At two-year periods, subjects returned and were asked to detail their medical history, demographics were measured, and in 50%, a further CT and cMRI were performed.

Since conception, the MESA study has produced a considerable volume of work detailing some of the functional and structural differences found in the left and right heart, between ethnic and gender groups both at baseline, with increasing age and in cases of obesity (Ventetuolo et al., 2011, Kawut et al., 2011, Chahal et al., 2012a, Aaron et al., 2011).

One such study, using cMRI to assess subclinical myocardial function by peak systolic strain (%) (PSS) and strain rate (1/s) (PSR) (for the LV) is discussed (Fernandes et al., 2011). In a group of 1099 individuals from the aforementioned populations, cMRI results demonstrated that African-Americans had lower PSS across more regions of the LV compared to other groups, whilst Chinese-Americans (the Asian-American group) demonstrated the highest PSS and PSR. Multiple regression modelling using an extensive list of confounders was performed in an effort to control for additional extrinsic influences. This demonstrated significant differences in the anterior, lateral and inferior walls of the African-American group compared to the referent Chinese-American group.

The majority of the work published by the MESA group focuses on LV function and uses both cMRI and CT as the method of assessment. A sub branch, the MESA Right Ventricle Study, has resulted in a number of key publications which have identified variation in RV size or function associated with obesity (Chahal et al., 2012a), exercise capacity (Aaron et al., 2011) and sex hormones (Ventetuolo et al., 2011).

Overall, the MESA study provides a basis from which this thesis can report and compare any associated changes found with echocardiography, from a different sample group. The use of echocardiography in different ethnic groups, for the purpose of cardiac assessment appears to vary between the LV and RV. Despite ethnic differences being discovered in the LV (Galasko et al., 2005) (Kizer et al., 2004) (Sharp et al., 2008) outside of the MESA group, studies focused on RV assessment appear to be limited.

(Chahal et al., 2010) performed echocardiograms on 458 healthy subjects, randomly selected from the LOLIPOP study in an effort to better understand the effects that ethnic based demographic factors have on LV measurements. Recruiting both UK based Indian and European healthy subjects the results suggest that European volunteers demonstrated larger LV volumes, LV mass and left atrial (LA) volumes even after indexing to body size (Chahal et al., 2010)

Differences in LV myocardial thickness and LV chamber size have been shown to exist between white and non-white athletes of similar training (Basavarajaiah et al., 2008, Kizer et al., 2004). However a recent study into the adaptation of the RV during endurance exercise demonstrated only minimal differences in size between both black and white athletes with both groups showing increased levels of RV hypertrophy compared to the control group. These changes suggested a physiological adaptation to high-level exercise, rather than a genetic adaptation, previously unreported in this particular group (Zaidi et al., 2013). In contrast, subjects from a non-athletic group within the MESA study did demonstrate changes in RV mass, volumes and RVEF, assessed using cMRI.

Importantly, these findings were independent of LV size and of adjustment for body size, using allometrically scaled processes (Kawut et al., 2011). The information regarding the ethnic based differences in the LV is unclear. Both method of assessment and the potential for athletic conditioning appear to influence results. What is clear is that limited normative data on the assessment of the RV exists within a healthy group of volunteers without the confounding influences of athletic performance or hypertension.

1.8. Age Related Changes in Cardiac Size & Function

Age related changes in cardiac size and function have been well documented and extensively studied. Changes can occur with pre-clinical disease, but also in its absence, as an adaptive process to changes in normal homeostasis within the human body (Oxenham and Sharpe, 2003). Several key cardiovascular changes associated with cardiomyocyte apoptosis (Centurione et al., 2002) are found to occur within the myocardium and arterial walls with increasing age, (Oxenham and Sharpe, 2003).

Increasing arterial wall stiffness subsequently results in a reduction in compliance, the effect of which is increased systolic blood pressure and afterload resulting in a myocardial adaptive process (Oxenham and Sharpe, 2003). Reductions in the rate of systolic distension, assessed via cMRI, showed this not only decreased with age but also varied between male and females typically decreasing per decade, from 30 years old in males (Aquaro et al., 2013). The effects are also evident with advanced age, and increasing ascending aorta diameters (Mirea et al., 2013).

The results of advanced age typically can affect the LV in a number of ways due to the loss of myocytes and an increase in the chamber to wall thickness ratio. Cellular apoptosis is considered a necessary process for the removal of damaged or unnecessary cells. Cardiomyocytes, however are considered to be long lasting, sharing, on average, the same lifespan as the organism, but without the same

regeneration profile as other cells (Shih et al., 2011). The result of damage to these cells is, therefore, potentially extensive or, in many cases, total.

1.9.RV changes with age

Early studies into RV Doppler derived indices from healthy subjects noted a change in both systolic and diastolic function, with increasing age, with a reduction in longitudinal systolic function (S' prime) and similar age related changes with both early (E') and late (A') diastolic velocities (Kukulski et al., 2000a).

Despite the small sample size, (n=32), older subjects (40-76 years, 13 men and 2 women) demonstrated reduced longitudinal systolic velocities compared to younger participants, but increased systolic short axis velocities, whilst diastolic function showed age related changes similar to the LV (Kukulski et al., 2000a).

This reduction in RV long axis function was further highlighted with a negative correlation between TAPSE and age (Tamborini et al., 2010). Similar changes within the LV, with a reduction in longitudinal function but increased EF, have also been demonstrated, using cMRI in older subjects (Nikitin et al., 2006).

These anticipated changes may result from myocyte apoptosis, the process of which, is masked from visual assessment in the 'normal' ageing heart by maintaining a compensatory, normal ejection fraction range; however, the diastolic changes associated with age are more frequently reported.

A prospective cMRI study conducted by Maceira et al in 2006, using a sample of 120 healthy volunteers, assessed reference values indexed to BSA using simple ratio metric scaling for RV volumes. Volunteers consisted of 60 men and women ranging in age from 20 to 80 years. Age, gender and BSA were all found significantly to influence both the volume and function of the RV (Maceira et al., 2006b).

Results suggested that, with an increase in age, both absolute and normalised RV volumes reduced whilst the RV ejection fraction increased, similar to that found within the LV. Age related diastolic variables displayed a similar trend to that of the LV. With increasing age, the early diastolic peak filling rate decreased whilst late peak filling rate increased (Maceira et al., 2006b).

1.10. Gender Related Changes

The allocation of cardiac myocytes at birth, between men and women, has been described as one potential cause for gender-based differences with increased LV mass seen to develop in men from puberty to adulthood (de Simone et al., 1995). Little is known about the development of the RV with regards to increasing gender disparity, however, women still tend to display a higher RV ejection fraction (RVEF) (Kawut et al., 2009). The echocardiographical assessment of the RV within this group, therefore, may be determined by both dimensional and functional parameters such as strain and strain rate.

Oestrogen levels in pre-menopausal women are known to provide protective therapy against LV heart failure and systemic vascular disease (Ventetuolo et al., 2011). Despite this, women have an increased risk of developing idiopathic PAH compared to men, with results from one study showing a 4.1:1 ratio of women to men with idiopathic PH (Badesch et al., 2010).

Gender differences in cardiac size (D'Oronzio et al., 2012, Willis et al., 2012, Maceira et al., 2006b) and in function (Kawut et al., 2011, Ventetuolo et al., 2011) have been demonstrated using a range of echo based assessment methods, within the RV.

A recent retrospective study demonstrated gender based differences in the right atrium (RA), across the long and short axis, and in the RV short axis dimension (D'Oronzio et al., 2012). RV end systolic area (RVESA) and RV end diastolic

area (RVEDA) were also measured and used to calculate RV fractional area change (RVFAC). In addition, TAPSE was assessed as a second measure of systolic function. Results demonstrated smaller chamber dimensions for women. After simple ratio metric scaling to BSA, using an y= a/x approach, where y equals the ratio-scaled interval, a equals the respective RV measurement and x equals the indexing variable, for example BSA; gender differences remained, advocating the use of indexed and non-indexed results, stratified by gender.

In agreement with the above study, work recently conducted by this group, found similar gender based differences, in a prospective study carried out to assess RV dimensions (Willis et al., 2012). Because this study was prospective in design, a detailed assessment of the RV was conducted at the acquisition stage, enabling a more thorough assessment of both cardiac size and function using ASE recommended methods and providing data that are more reproducible in future studies. Inclusion and acknowledgement of gender based differences remains a clinically important factor when attempting better to understand cardiac chamber evaluation.

1.11. RV Anatomy

Anatomically the RV is the most anterior situated cardiac chamber lying immediately behind the sternum (Ho and Nihoyannopoulos, 2006) and anteriorly positioned to the LV (Lindqvist et al., 2008). In the absence of any gross congenital heart conditions, the RV is enclosed by both the tricuspid and pulmonary valves.

The LV, under normal conditions, develops from birth to form an ellipsoid shaped chamber with thick muscular walls. The RV by comparison is thin walled and crescent shaped (Foale et al., 1986). Although from the apical four-chamber echocardiographic view the RV appears to be smaller than the LV, RV volume is in fact very similar. Results from cMRI studies indicate that on average the RV end diastolic volume (RVEDV) is 49 – 101 mL/m2 compared to 44 – 81mL/m2 for the LV (Lorenz et al., 1999)

The study of the RV has often been restricted due to the complex nature of the chamber and, in part, to limitations in the imaging modalities that have been available. As the techniques for imaging have improved, however, there has been a resurgence in research aiming to quantify both the function and volume of the RV in various disease states (Niemann et al., 2007).

The importance of RV assessment has often been over-shadowed by that of the LV due to the LVs greater muscular mass and the potential to cause patient symptoms.

The role of the RV is to generate flow more so than high pressures thus making its functional pumping assessment more difficult to evaluate. Unlike the LV, which has clear patterns of myocardial thickening, seen from multiple views, the RV due to the bellows-like action of the chamber remains harder to quantify with its less dramatic contraction and relaxation.

In addition, the right side and left side of the heart are linked in both diastolic interactions and systolic interactions ensuring that complete assessment of one

should also be matched with complete assessment of the other. This relationship known as interdependence has become more prominent with the increased interest in disease states such as pulmonary hypertension (Magder, 2007).

1.11.1. RV hemodynamic.

In cases of myocardial infarction, the LV is heavily scrutinised for any regional wall motion abnormalities secondary to damage caused to the myocardium. In cases of inferior infarction, however, 40 – 50% of patients have some degree of RV involvement that may result in hemodynamic compromise and a poor clinical outcome (Lindqvist et al., 2008).

RV function remains a major determinate of symptoms and exercise capacity in heart failure patients when compared to LV function alone (Baker et al., 1984) and this has been attributed to a depressed RV long axis function (Ghio et al., 2001). The RV as a unit operates at much lower pressures. As a result, it has developed a thinner more mobile free wall compared to the LV.

The dominance of the LV over the RV creates the sepal contour that gives the RV its crescent shape, with a concave sepal position towards the LV in both systole and diastole under normal loading conditions (Vitarelli and Terzano, 2009).

This lower pressure system results in a muscle mass that is approximately one-sixth that of the LV. This is due in part, to the different preload and after-load conditions they both experience during the normal cardiac cycle. The thin walled RV however, still ejects the same rate and volume of blood as the more muscular LV. This is typically due to the physiology of the pulmonary circulation compared to the systemic circulation, with right sided circulation pumping into a much lower pressure system (Dell'Italia, 1991)

Because the RV and LV are connected in series, bar any significant valvular abnormalities or cardiac shunt, the stroke volume of the RV will normally match that of the LV (Haddad et al.). This, combined with larger end diastolic volumes (RVEDV), ensures that the RV ejection fraction is lower, with typical ranges from 40 - 45% compared to 50 – 55% for the LV (Lang et al., 2005).

1.11.2. RV structure

Traditionally the RV has been described as having two main components. Despite this, for the purpose of complex congenital heart disease, three are often described: 1) The inlet, which consists of the tricuspid valve, chordae tendineae and papillary muscles; 2) The trabeculated apical myocardium; 3) The infundibulum, or conus, which corresponds to the smooth myocardial outflow region (Haddad et al., 2008).

Anatomically the RV is thus divided into two parts by a thick intracavity muscle band known as the crista supraventricularis (CSV) (Dell'Italia, 1991). This band has been linked to the systolic function of the RV (James, 1985) and contains elements of the electrical conduction system known as the right bundle branch within its structure.

Adjoined to the CSV is the septomarginal trabecula, more commonly known as the moderator band. Originating from the lower segment of the CSV, the moderator band is often visibly separate from the surface of the interventricular septum, attaching to the anterior free wall (Kosinski et al., 2007).

The CSV is described as resembling a bracing strut. During systole it serves a dual purpose to both narrow the tricuspid orifice and to bring the freewall of the RV toward the septum. This process assists in the emptying of the ventricle in to the pulmonary artery. Due to the more anterior position of the CSV, the clockwise rotation of the LV apex (forming part of the torsion squeeze) acts by

pulling in the RV freewall. Conversely, in diastole, the hollow structure is hypothesised to assist in the opening of the RV by pushing out the freewall.

The attachment of the CSV to the septum allows it to act as a connection to the LV and thus enhance the integrated actions of both ventricles (James, 1985) described as ventricular interdependence. The CSV is the only attachment from the central upper portion of the RV freewall, and may help to synchronise the timing of the freewall to the septum and subsequently the LV (James, 1985). This association can account for 20% to 40% of the pressure generated within the RV through the systolic forces of the LV (Santamore and Dell'Italia, 1998).

This interaction between the ventricles could help to explain how the RV is able to maintain adequate pressures levels (through the assisted pull of the LV). Canine models have demonstrated that even in the event of a severely damaged RV free wall through techniques such as electrical cautery (producing on average over 75% RV necrosis) adequate RV pressures were maintained. When the LV function was moderately damaged in a similar fashion, however, the animals quickly succumbed to shock (Bakos, 1950).

Ventricular contraction cannot be assessed as if both ventricles were a single unit. Typically RV contraction is characterized by a reduction in length, due to the predominance of longitudinal fibres, with the width at the mid-point varying only slightly, while the LV displays a change in both length and width with an oblique movement toward the apex and towards the interventricular septum (Rushmer et al., 1953). (Rushmer et al., 1953) also demonstrated differences in the displacement of the epicardial and endocardial surface with endocardial displacement greater than epicardial and more pronounced at the base of the RV. This may have implications on techniques such as STE which rely on tracking myocardial movement between both these surfaces and on a segmental level.

1.11.3. Myofibre Architecture

Both the left and right ventricle are composed of complex layers of fibres designed to aid both the systolic and diastolic function of the chamber. This complex network of fibres has been described in various levels of detail for over 300 years (Greenbaum et al., 1981). The myocardium is comprised of this three dimensional array of fibres consisting of myocytes. Each of the myocytes that make up this complex array are joined at either end but also at the side branches.

It is now known that the orientation of these fibres differs between the left and right ventricle (Ho and Nihoyannopoulos, 2006). The LV architecture is comprised of radial, circumferential, and longitudinal fibres with the circumferential components present particularly in the mid-wall and the base of the LV (Henein and Gibson, 1999).

The longitudinal component of the LV is found in the deeper subendocardial fibres and subepicardial fibres of the freewall and alongside the papillary muscle (Greenbaum et al., 1981). The subepicardial fibres of the RV are predominantly arranged in a circumferential fashion, parallel to the atrioventricular groove and encircling the subpulmonary infundibulum. These superficial fibres are linked across into the LV forming part of the bond that joins the LV and RV together (Ho and Nihoyannopoulos, 2006).

The RV muscular wall is only 3-5mm thick (Foale et al., 1986) and within this there is room for subepicardial and subendocardial layers comprising of circumferential and longitudinal fibres only. Studies which have compared both normal RV architecture and congenital abnormalities such as tetralogy of Fallot, however, have discovered that, in cases of RV hypertrophy, which is common to tetralogy of Fallot, a third layer was present between the subepicardial and subendocardial layers, composed of circular fibres giving a similar structure to that of the LV (Sanchez-Quintana et al., 1996).

Other examples of the musculature adaptation of the RV can be seen in congenital patients in whom complete transposition of the great arteries results in

a switch of the great vessels at either atria or great arterial level (Davlouros et al., 2006). This results in the RV supporting the systemic circulation, leading to long-term concerns regarding the onset of RV failure, arrhythmias and compromised long term quality of life (Davlouros et al., 2006)

1.12. Echocardiographic assessment of the RV

1.12.1. Imaging History

In order to understand the newer techniques that have been developed and to appreciate the importance of 2D measurements, it is imperative that the benefits and limitations of several common techniques are discussed.

Edler and Hertz are credited with being the first to use ultrasound for the purpose of cardiac examination (Fraser, 2001). The technique used was called A-mode (amplitude-mode) and displayed reflected signals at representative depths within the cardiac chamber. Rapid send and receive signals were created which aided in the identification of fast moving structures such as valves based on the timing and amplitude of the signal. With the application of time shown on the horizontal axis and with each amplitude signal converted into a grey-scale level this technique developed into M-mode (motion mode) that is more commonly used today (Otto, 2004).

The M-mode technique encompassed very high sampling rates giving added benefit to the sampling of valves and fast moving structures. Further validation of this technique using autopsy samples quantified M-Mode as an accurate method for the assessment of chamber size within a symmetrical ventricle (Devereux and Reichek, 1977, Devereux et al., 1986).

Due to the single plane nature of the measurement, however, regional distortion of the ventricle due to infarction or other conditions could result in inaccurate measurements (Lang et al., 2005). The continued use of M-mode imaging lead to the development of mathematical assumptions based on LV chamber size for the calculation of LV volume (Teichholz et al., 1976).

With LV volumes thereby established, calculation of LV ejection fraction shortly followed. These same assumptions of volume could not be applied to the RV however, given its asymmetric shape, thus creating the first disparity in assessment between the LV and RV. Other imaging modalities present at this time such as cMRI and CT, used either fixed transverse or saggital orientations that were dependant on external landmarks. This resulted in oblique cuts across the asymmetric RV that proved difficult to standardise between patients and thus quantify (Foale et al., 1986).

Further development of this technique saw the ultrasound beam change from static to a rapid sweeping position across the cardiac chamber. This movement, generated either mechanically or electronically, saw the introduction of 2D live imaging of the cardiac chambers. The advantage being beat by beat display and the possibility of multiple tomographic views of all cardiac chambers (Otto, 2004).

1.12.2. 2D Imaging

2D and M-mode assessment of the RV is complicated by the complex geometry of the chamber (Jurcut et al., 2010). Sir Magdi Yacoub described the structure of the LV as a "flask" with a conical shape, which contains one inlet and one outlet within the one chamber. This 'design' allows for a bolus of blood to be delivered against a high-pressure system. The RV, on the other hand, is a flattened tube that wraps around the LV with separate inlet and outlet orifices designed for pumping blood against a lower pressure system (Yacoub, 1995).

Because of the complex shape of the RV, which in the normal heart assumes a crescent shape from the short axis, and a triangle from the frontal aspect, multiple views, each characterised by specific landmarks must be used (Ho and Nihoyannopoulos, 2006).

Three traditional acoustic windows are used to assess the RV: parasternal, apical and subcostal views. Subdivided within these windows, images are recorded that allow adequate examination of each of the RV components. Further details are discussed in section 2.7.

1.13. Influential papers in RV assessment

The assessment of the RV has been studied extensively using echocardiographic techniques for many years. Within that time, three key papers have been published that have resulted in either changes to or reinforcement of, good assessment techniques of the right heart.

This does not represent an exhaustive list of research available on the RV but the importance of these three papers as a chronological timeline for RV assessment within the wider community should be appreciated.

1.13.1. Echocardiographic measurement of the normal adult right ventricle

The work conducted by Foale and colleagues in 1986 (Foale et al., 1986) remained, until 2010, the main standard reference for normal RV dimensions used by both EAE and ASE guidelines (Lang et al., 2005). The study group consisted of 41 healthy volunteers (19 – 46 years) with adequate technical quality images. Having identified the three regions of interest within the RV (inflow, outflow and body) a standardised protocol was devised.

This detailed protocol consisted of twelve 2D calliper chamber measurements and 10 wall thickness measurements. Four of these measurements were made in the apical four-chamber view. This equated to two measurements within the inflow section of the RV (tricuspid valve annulus and basal third), one measuring the body of the RV within the middle third at the widest dimension, and one measuring the annulus to apex length of the RV.

Calculated intraobserver and interobserver variability was reported in order to confirm the accuracy of each measurement. Results were expressed both as a range of absolute differences but also as a percentage difference between the two (Foale et al., 1986).

The aim of this paper remained simply to quantify RV size within the pre-selected normal group. No reference to gender or age stratification was made within the aims and objectives, or within the results and the indexed measurements provided by Foale et al. were never fully adopted into mainstream routine assessment with the exception of some surgical application.

For example, assessment of the TV annulus in the work-up for a TV replacement or annuloplasty will involve annular measurements indexed to body size (Dreyfus et al., 2005). The tissue that comprises the annular ring could determine the ability of this area to dilate and change with respect to alteration in body geometry. Unlike many of the other RV measurements however, the annular diameter encompasses rigid structures that carry both structural and functional consequences should they dilate, such as valvular prolapse or pathological regurgitation.

1.13.2. Recommendations for chamber quantification.

Despite the small sample size used in Foale's paper, its use in guidelines, published in 2005 suggested that for almost twenty years there had been a lack of research interest in-to developing and expanding the assessment of the normal RV.

This was compounded by a disparity between LV and RV assessment as a whole. Nevertheless, the importance of standard RV linear dimensions was stressed within this document as forming a key part of the chamber assessment (Lang et al., 2005), and that still remains the case today (Rudski et al., 2010).

Enhancements within the 2005 guidelines brought about the introduction of grading for RV dilatation. Mild, moderate and severe RV dimensions were calculated using two, three and four standard deviations from the mean. Five measurements of the RV were suggested. Three made in the apical four-chamber view and two in the RVOT and parasternal short axis (aortic valve level). Although these were referenced to Foale et al.'s work, the location of the measurements appeared to have been simplified with the location of RVD-2 described as being measured

"…at the level of LV papillary muscle" p1451 (Lang et al., 2005).

Foale and colleagues provided clear anatomical descriptions that could be used to easily replicate their measurements. However, the inclusion of anatomical landmarks such as the LV papillary muscle, may have aimed to ensure a better degree of standardisation between studies. Despite this suggestion by Lang and colleagues and acknowledging this reference point, practical assessment from the focused RV view dictated that RVD-2 measurements within this thesis be made at the mid cavity level. In support of this and Foales guidelines, work by Triulzi et al (1984) also identified the location of RVD-2 as a mid-cavity measurement in a group of healthy volunteers.

The 2005 guidelines, which almost exclusively used the work of Foale and colleagues, were validated against cardiac MRI in 2008, in a group of normal (n = 31), repaired Tetralogy of Fallots (n = 33) and atrial septal defect/PAPVC (n = 23) patients. 2D measurements (cm) of the RV were made on both MRI samples and echo images as well as MRI derived RV volumes (ml). (Lai et al., 2008)

This study revealed limitations to the current 2D measurements with results on average underestimating RV size in comparison to MRI (Lai et al.). Results suggested a weak correlation between both MRI calculated dimensions and echo-derived dimensions with RV cMRI volumes. Only cMRI derived measurements of the apical images were made however. Despite the correlations between these two measurement techniques being weak, this was based on comparisons

between cMRI derived volumes and RV 2D calliper measurements made in the apical views, RVOT and Pulmonary Artery (PA).

The suggestion regarding echocardiographic assessment is that these areas should be treated as three separate components (Ho, 2006, Foale et al., 1986, Haddad et al., 2008) and hence the orientation of the inflow and outflow may not lend itself to comparison with the RV body.

The methodology employed by Lang et al (2005) to calculate the reference ranges and subsequent grading of mild moderate and severe, was based on the use of standard deviations derived from the original study source (Lang et al., 2005). The reference ranges for the normal study sample were calculated and then according to a set number of standard deviations, increased appropriately to encompass one (mild); two (moderate) and three (severe) reference ranges.Lang and colleagues noted the limitations to their method based on the assumption of sample normality and the subsequent effect this will have on standard deviations. As a review paper, they were unlikely to have access to the raw study data.

Sample size remains critical to the calculation of reference ranges with several small samples made from within the same healthy population resulting in varying 95% reference intervals (Altman, 1991). With this potential source of error, and the fact that the sampling was derived from different populations of healthy individuals, the calculation of varying degrees of RV dilation within this study has been removed from the 2010 guidelines to be replaced with a simple RV maximum dimension. Based on this rationale it was felt that, despite the larger sample size, it was not best practice to recalculate these pathological ranges with the study results.

1.13.3. ASE guidelines for RV assessment.

In 2010 a meta-analysis, published by the American Society of Echocardiography (ASE), undertook a systematic review of right ventricular size

and function (Rudski et al., 2010). In this review, questions were raised over the progression of RV assessment, relative to that of the LV, and the lack of normative data for techniques such as speckle tracking echocardiography (STE) and 3D volume analysis (Rudski et al., 2010).

During the course of this research, the recognition of the need for new RV guidelines resulted in the 2010 ASE paper which detailed the requirements for a full and comprehensive assessment of the RV (Rudski et al., 2010). Despite the increased number of healthy subjects for each of the parameters discussed, there was still a general agreement that further work needed to be undertaken within the normal population.

Results published within these guidelines were the culmination of several different studies (between 8 – 12 studies consisting of between 159 and 400 participants) in which RV measurements had been made. These measurements included RVD 1-3 and RVOT 1-2 following the same acquisition sequence as previously reported (Lang et al., 2005). Importantly however, in this paper the group acknowledged that the previous guidelines had:

'focused on the left heart, with only a small section covering right sided chambers'(p688) and with advances in the assessment of the right heart a *'greater dissemination of details regarding the standardisation of the RV echocardiographic assessment'* (p688) was required (Rudski et al., 2010).

New mean, upper and lower reference values and 95% confidence intervals were calculated. In addition, measurements were also made from the RVOT in the parasternal long axis view. In these guidelines, therefore, clear direction was given on the acquisition of RV images as well as the pitfalls surrounding the lack of fixed reference points for measurement.

It would appear, however that even in the updated guidelines, a failure to undertake the recommended exam suggested by (Foale et al., 1986) still raises doubts over the correct method to measure the RV, potentially explaining some of the reduced correlations with the gold standard cMRI (Lai et al., 2008).

The 2010 guidelines also pointed out very clearly that, given the retrospective nature of the study, the results presented from their review did not account for the impact of body size. Consideration should therefore be given to patients that are classified at either extreme of the suggested reference values (Rudski et al., 2010).

The publication of this document not only provides a justification for this project but also a good baseline to which the prospectively collected raw data within this study can be compared. Furthermore, it offers a valuable insight into the prospective differences between ethnic groups, and a detailed assessment of the reproducibility of these techniques within the arguably most common patient group.

1.14. Ratiometric and Allometric scaling.

The use of patient body size measurements in the assessment of cardiac dimensions has commonly taken place due to the suggestion that these are directly related. The calculation of data indexed to BSA or 'simple ratio scaling' is a common method for quantifying measurements, adapted to body size (Batterham et al., 1999).

Ratio-scaled or indexed measurements have been available for the LV and LA in standardised guidelines for almost a decade (Lang et al., 2005). However the use of ratio scaled results for the RV dimensions is currently lacking (Willis et al., 2012), (Rudski et al., 2010, Hoit, 2012).

A recent retrospective study published by D'Oronzio et al in late 2012 demonstrated the benefits of indexing to BSA, whilst also highlighting gender differences in RV and RA dimensions that existed within a large cohort of patients with normal echo studies (D'Oronzio et al., 2012).

Despite the apparent lack of indexed results, BSA has been documented to influence both RV and LV measurements (Maceira et al., 2006a, Greutmann et al., 2010, Maceira et al., 2006b) (Willis et al., 2012), is often helpful in quantifying measurements that fall outside upper reference limits (Lang et al., 2005) and in reducing gender differences (D'Oronzio et al., 2012).

Within this model it is assumed (by virtue of the technique) that the relationship between the physiological variable (in this case RV dimensions) and the body variable (for example, height), takes the form $y=a/x$ with the line of best fit passing through the origin (Neilan et al., 2009). Cardiac dimensions and volumes have demonstrated a strong relationship to body size (Neilan et al.). However, this assumes a size independent relationship between y and x.

This simple ratiometric scaling method uses a measure of body size (in this case BSA) and divides it by a given dimension. The result, using the following formula, is a linear relationship between the two assigned parameters $y=a/x$ where y is the resultant parameter, a is the absolute dimension and x is the calculated BSA (Hoit, 2012). 2005 guidelines provided jointly by the ASE and EAE (now renamed the European Association of Cardiovascular Imaging (EACVI)) provide normative ranges for all chamber measurements in addition to LV indexed measurements (Lang et al., 2005).

The ability to calculate measurements that are size independent is essential when distinguishing potential physiology from pathology (Oxborough et al., 2012b). The calculation of BSA itself is not without problems. The commonly used Du Bois formula (Du Bois D Fau - Du Bois and Du Bois, 1916) has been shown to underestimate BSA in clinically obese people (Livingston and Lee, 2001).

Calculated BSA ratio scaled measurements of cardiac dimensions assume a direct linear relationship. As BSA, (a component of height and weight) increases so will cardiac dimensions. As a simple method of assessment, this may be applicable to a large number of people who are of average height and weight and for whom, absolute measurements may suffice. Oxborough et al (2012) recently

demonstrated a non-liner relationship within certain healthy groups with cardiac dimensions measured in excess of normal ranges (Oxborough et al., 2012b).

All participants were ‘well’ and classed as athletes based on the number of hours per week of high intensity training they performed. Several measurements of the RV were made which demonstrated that within this ‘well’ group between 40-69% of cardiac dimensions exceeded those normal limits. BSA alone was not sufficient to produce size independence within this group, demonstrated by the significant correlation between RV measurement and BSA, post indexing therefore failing to elicit complete independence (Oxborough et al., 2012b). In addition, when assessed using Tanner ‘special circumstance’, (TSC) (described in section 2.11) these measurements failed to conform (Oxborough et al., 2012b). With this group of well, highly trained endurance athletes, allometric scaling proved to be more appropriate, providing a size independent measurement specific to that particular group. Similar results were also found using junior athletes, with the suggestion that these studies need to expand to included not only healthy volunteers but also subjects from different populations (George et al., 2001).

Height and body mass have formed the basis for many studies involving body size because of the ease with which they can be calculated with a high degree of accuracy (Batterham et al., 1999). However the inclusion of body mass has been subject to some discussion given assumptions that must then be made about its distribution between muscle mass and adipose tissue (Batterham et al., 1999). Fat free mass has been suggested as a suitable alternative that accounts for the distribution between adipose and muscle mass. Despite this, it is impractical to use this method in daily clinical practice.

The process of allometric scaling refers to one possible method for the scaling of non-linear dimensions using a specific scaling beta (b) exponent. This is calculated using a specific scaling factor, for example BSA. Before the calculation of this exponent however, the assumption of a non-linear relationship must be proved. This is achieved by assessing the data for conformity to TSC which is rarely satisfied in biological models (Batterham et al., 1999). The

details surrounding the calculation of both the beta value and TSC, can be found within the methods section.2.11.

Currently, together with a lack of ratio scaled (indexed) measurements to BSA for the RV, there is also a lack of normative non-linear scaled data (Hoit, 2012). A recent study conducted by D'Andrea et al (2012) further demonstrated this disparity between both normal and athletic subjects, by highlighting the difference in RV dimensions between healthy volunteers, strength trained athletes and endurance trained athletes. Despite the inclusion of ratio-scaled RV parameters to BSA, this was insufficient to produce body size independent RV volumes. As a result, the authors sought to employ allometric scaling to 3D RV volumes. Again, endurance athletes demonstrated increased RV volumes compared to control and strength-trained athletes (D'Andrea et al., 2012).

The current evidence base for the use of allometric scaling appears to feature subjects that are either very young (and therefore may not subject to normal adult examinations) or undertake high or professional levels of sport-related training. The result is studies featuring linear or area measurements, that are at the upper limits or exceeding normal reference ranges, and it is here that allometric and traditional ratio scaling are helpful. As a result, the data collected within these studies can only be applied to an athletic population matching the training level and intensity of those studied.

Both Batterham (Batterham et al., 1999) and Oxborough (Oxborough et al., 2012b) suggest the need for allometric data to be added to the reference ranges for the non-athletic population, documenting the body size independent scaled ranges. Establishing these differences between essentially healthy control subjects and then calculating the exponent required to normalise these measurements provides a helpful basis for the assessment of pathological changes to the RV.

The application of non-linear scaled data in conditions such as ARVC could help to ascertain normal physiological conditioning of the RV as opposed to an inherited cardiomyopathy (Oxborough et al., 2012b). The ease at which ratio

scaled data can be obtained does suggest that there is still a place for the use of BSA linked measurements. It is also pertinent, however to discuss the possibilities of measurements that are ratio linked via the process of allometric scaling in groups of healthy volunteers from varying ethnic backgrounds.

1.15. Functional Assessment

Functional assessment of the RV has improved over the last two decades due in part to the improvements in cMRI as a reference source and in the development of newer techniques that allow functional capacity to be assessed. A number of these are discussed within this section with a more detailed assessment of both STE and 3D volume assessment in sections 1.16 and 1.17.

Given the musculature of the RV, a recent review of cMRI tagging studies confirmed that shortening of the RV is greater longitudinally than radial (Petitjean et al., 2005). As a result, appropriate functional assessment techniques must be selected. Qualitative assessment via 2D echocardiography is adequate in the clinically normal patient, but if more quantitative assessment is required, in particular regional assessment as opposed to just surrogate global markers, then conventional echocardiography can prove limited (Bleeker et al., 2007).

Both invasive and non-invasive techniques exist to assess systolic and diastolic function of both the left and right ventricles. Each method has its own inherent limitations. Assessment of cardiac function can take place at a global level or segmentally, depending on the chamber assessed and the information required. Typically, a 16 segment with an alternative 17 segment model is used from which regional (or segmental) assessment can be made (Schiller et al., 1989, Cerqueira et al., 2002).

Adequate function of the RV is imperative to ensure good pulmonary perfusion and low systemic venous pressure, preventing tissue damage and organ congestion (Bleeker et al.). RV contraction does not occur in the same way as the

LV. This combined with the asymmetric shape, variation in myocardial structure and limited views ensure that the RV cannot be assessed using many of the traditional LV functional markers.

1.15.1. RV Fractional Area of Change

Ejection fraction (EF) is one of the commonly used measures of systolic function. Calculated as the stroke volume expressed as a percentage of the end diastolic volume (Bleeker et al., 2007) this method has in the past been calculated via M-mode measurements but this is no longer recommended due to the restricted anatomical assumptions made regarding chamber dimensions and shape (Lang et al., 2005).

There is some suggestion that with experience EF can be estimated by eye alone (Hope et al., 2003). Because of the risk of interobserver variability and the experience required to make such an assessment, visual assessment must be used in conjunction with other methods (Lang et al., 2005). Importantly, however, systolic performance can be affected by factors such as preload, afterload, contractile state, dyssynchrony and heart rate (Mahler et al., 1975). For the RV, ventricular interdependence is also a factor resulting from any adverse systolic or diastolic interactions via the ventricular septum and pericardium (Santamore and Dell'Italia, 1998).

Unlike the LV which can be assumed to be an ellipsoid shape and can be traced in a number of views, the RV presents more of a challenge. Conventional biplane methods of calculating EF are more difficult to apply to the RV owing to its complex shape (Lindqvist et al., 2008). Surrogate markers of RV EF such as RV fractional area of change (RVFAC) have been compared with cMRI with (Schenk et al., 2000) (Anavekar et al., 2007) with favourable results. RVFAC, measured in the apical four-chamber view, expresses the change in RV area from end-diastole to end-systole as a percentage. The limitation to this and any other 2D based method is the requirement for good endocardial border definition.

1.15.2. Tricuspid Annular Systolic Plane Excursion

TAPSE remains the required minimum for RV assessment. Utilising M-mode, a cursor can be placed in the lateral tricuspid annulus within the apical four chamber. Acknowledging the longitudinal motion of the tricuspid annulus towards the apex, a calliper measurement can be made from the base of the M-mode trace to the peak.

This represents a shortening of the longitudinal fibres found within the RV. As a result, this is used as a surrogate marker of global RV function. As with other regional methods it assumes that displacement of the basal segments is a true representation of global RV function despite only being measured in the inlet portion (Rudski et al., 2010).

Validation studies of TAPSE with radionuclide angiographic derived RVEF showed a strong ($r=0.92$ $p<0.001$) correlation (Kaul et al., 1984). When compared to RV EF derived by Simpsons method, however, a weaker, but still significant ($r=0.48$ $p = <0.001$) correlation was observed (Miller et al., 2004). This is most likely due to the previously described limitations of calculating RVEF. Despite this positive correlation, some studies have found there was no correlation of cMRI derived RVEF and TAPSE ($r = 0.17$ $p = 0.30$) (Anavekar et al., 2007).

RV guidelines (2010) pooled together more than 40 studies totalling over 2000 patients. One study in particular examined 900 patients, 150 normal aged matched controls and 750 with various conditions. This study identified a TAPSE cut-off value of 17mm and despite a low sensitivity a high specificity was noted though not reported (Tamborini et al., 2007). Lower reference values for TAPSE were given as <17mm (Rudski et al., 2010).

Despite the comparable results to cMRI there remains a need for additional assessment methods that will remain load independent and able to tackle the complex geometry of the RV.

1.15.3. Tissue Doppler Imaging

Based on Doppler principles, TDI is a versatile, quantitative technique that provides information on the velocity of tissue movement throughout the cardiac cycle (Bleeker et al., 2007).

TDI provides an objective assessment of both global and regional myocardial performance. By utilising pulse wave signals, previously adapted to remove myocardial 'artefact' and by reducing the band pass filter and subsequently the pulse repetition frequency, it is possible to assess longitudinal movement of the myocardium reported as cm/sec (Sutherland et al., 1999).

Often studied at either the mitral or tricuspid valve annulus, this assessment of longitudinal function remains important for both the left and right ventricles given the strong association between longitudinal function and subsequent global function (Sutherland et al., 2004). The dominance of longitudinal fibres within the RV (Ho and Nihoyannopoulos, 2006) means that any reduction in their contractility could result in significant symptomatic problems. Assessment of annular measurements lends itself to good alignment through the ultrasound beam and has been extensively studied in both healthy volunteers (Chahal et al., 2012b) and pathological conditions such as pulmonary embolism (Dentali et al., 2013) and PH (Lammers et al., 2012).

Calculations of normal TDI parameters have been derived from a large population based study featuring healthy volunteers from a wide age range. The Umea General Population Heart Study in Sweden found a mean value of approximately 15cm/s at the annulus and basal segment and remained age-independent despite age-related changes in other Doppler measurements (Lindqvist et al.). The 2010 RV guidelines, found a lower reference value for normal hearts of 10cm/s (95% CI's 9-11 cm/s) based on the pooling of 43 studies (*n* 2139) (Rudski et al., 2010). This value was subsequently chosen as the minimum reference value for normal volunteers within this study.

Velocities can be displayed as either a pulsed wave (PW) spectrogram (figure 1.0) or as a colour-coded image suitable for offline analysis. The traces provided by the PW TDI signal give a clear indication of the myocardial velocities throughout the cardiac cycle. Systolic (S'), early diastolic (E') and late diastolic (A') velocities are available from any myocardial wall segment enhancing regional assessment of chamber function.

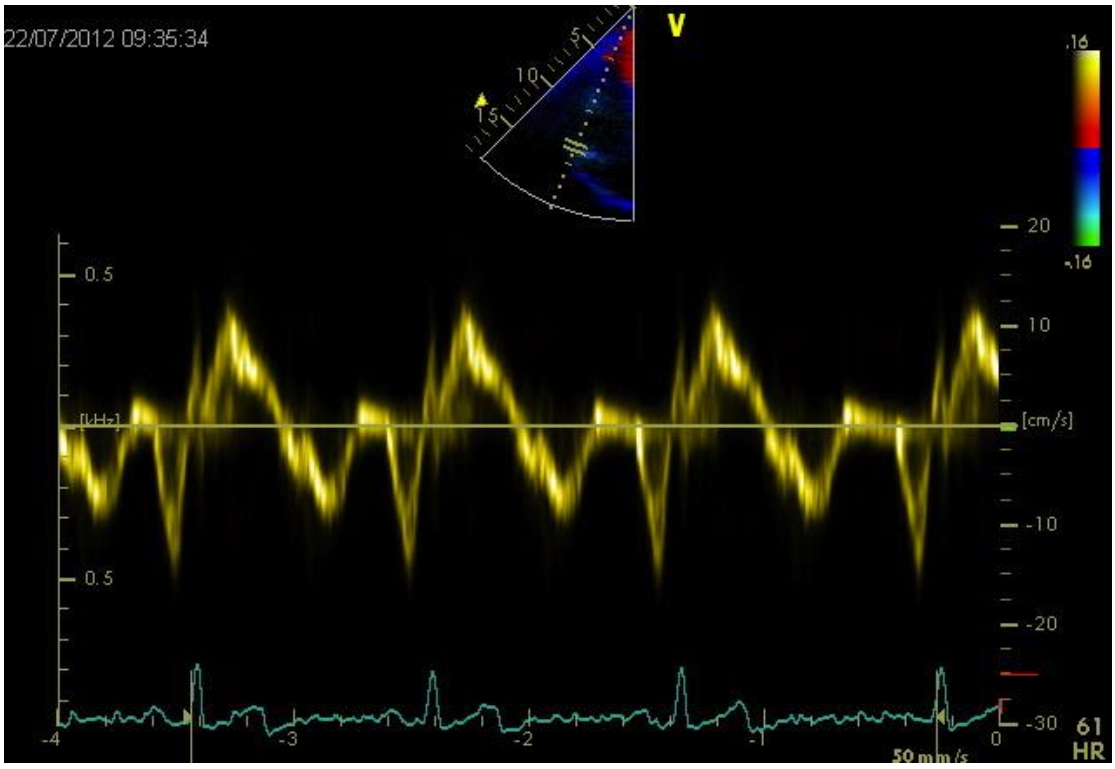


Figure 1.0 Pulsed Wave (PW) Tissue Doppler Imaging (TDI) signals derived from the basal lateral wall of the RV displaying instantaneous longitudinal velocity (cm/s) of focused myocardial movement throughout the cardiac cycle. See section 1.15.3

This technique is less dependent on chamber geometry and does not require clear endocardial border delineation to trace.

There has been a suggestion that this technique is suitable even in subjects with poor image quality (Jurcut et al., 2010) but both movement and artefact are known to influence the results (Horton et al., 2009). Despite the advantages, TDI is limited by the angle of intercept from which these measurements can be made with an angle of intercept >20° potentially leading to erroneous results due to the manner in which the fixed reference PW sample cannot track the mobile endocardium (Jurcut et al., 2010).

Despite its obvious versatility, TDI was first believed to be load independent. Further research however, has shown that preload can lead to significant variance in myocardial velocities and, as such, should be considered when assessing ventricular function (Oguzhan et al., 2005) (Andersen et al., 2004). Once the velocity of the tissue has been calculated, other parameters can be derived such as displacement, strain rate and strain.

With the obvious limitations in deriving a volume based 'model' that allows for quantification of RV function, TDI provides a simple solution given the anatomical considerations and the fact that the RV is predisposed to less definable endocardial borders. As a result, TDI measurements of the RV freewall are common place and form part of the recommended guidelines for RV assessment (Rudski et al., 2010).

Interestingly myocardial velocities were found to reduce when samples were made in both the mid and apical segments giving rise to the suggestion that there may be a gradient from the basal to apical segment. Other studies have identified a reduction in S' wave measurements in patients with heart failure compared to healthy controls.

Using a cut-off value of <11.5cm/sec Meluzin et al. were able to predict RV dysfunction (defined as <45% via RVEF via single photon emission computed tomography) with a sensitivity and specificity of 90% and 85% respectively using PW TDI (Meluzin et al., 2001).

Colour TDI provides an alternative approach to velocity imaging. Unlike PW Doppler TDI which displays maximum instantaneous velocities, colour VTI displays the average velocities within a specific region of interest (ROI) (Horton et al., 2009). This results in a reduction of approximately 25% lower systolic and diastolic velocities (Kukulski et al., 2000b).

The associated ROI can be shaped and defined in post processing unlike PW TVI, which is limited to the coverage of the PW sample window only. Colour TDI also has the benefit of sampling at multiple locations across the same

cardiac cycle. By carefully tracking the movement of the myocardium frame by frame; it is possible to detect regional movement throughout the cardiac cycle resulting in multiple average velocities. This process is time consuming but has allowed for a more accurate assessment of regional function to be established.

The underlying limitations with the technique and thus with the resulting calculation of PSS and PSR, are noise, poor reproducibility and the dependence on angle orientation (Castro et al., 2000). Studies comparing the reproducibility of these techniques have also highlighted clinically relevant interobserver and intraobserver reproducibility (Castro et al., 2000). These findings have limited the widespread use of this method and can be attributed to the limitation of single, 1D imaging.

The technique remains one dimensional because strain characteristics are only being calculated along the line of intercept, limiting use to mainly apical images in which longitudinal function can be assessed (Geyer et al., 2010).

1.15.4. Strain and Strain Rate

Strain and SR are measures of deformation, or changes in shape. Mirsky and Parmley discussed the concept as early as 1973, studying both normal and infracted animal models. (Mirsky and Parmley, 1973). In its simplest where only one form or direction of movement is assessed (i.e. one dimensional), strain describes the deformation of an object relative to its original length using the following formula:

Equation 1.0 Simple strain equation

$$\frac{L - L0}{L0}$$

At rest, an object that has an initial length (L0) can be stretched or compressed to a new length (L). The results of this are traditionally expressed as a percentage, with a negative score dictating a shortening in length. Should L equal L0 then strain remains zero (Mirsky and Parmley, 1973). As the special equivalent of velocity, SR can provide precise, segmental orientation of both normal and

diseased segments (Geyer et al., 2010). As a results it requires a high temporal resolution (>100Hz) so as to avoid underestimation due to under sampling (Geyer et al., 2010).

Strain indicates the amount of deformation, whilst strain rate, displays the speed at which it is taking place. So, despite two objects displaying the same percentage of deformation, the speed at which this occurs can vary. This relationship is similar to that derived from velocity and displacement (Heimdal, 2007).

How this deformation relationship is described can also vary depending on how the calculation of SR is made. Most commonly used is Langarian strain which describes motion around a specific (tethered) point, in this case the myocardium, as it moves through both time and space. This technique is also applied to tagged cMRI studies where end diastolic tissue dimensions represent the unstressed (0%) material length. From here any positive (lengthening) or negative (shortening) can be calculated throughout the cardiac cycle (Kowalski et al., 2001).

1.16. Speckle Tracking Echocardiography

The use of myocardial STE is a relatively new echocardiographic tool through which the assessment of myocardial function can be made. Traditional methods of assessing systolic function either relied on the sonographer's personal experience or were dependant on anatomical orientation, cardiac motion, cardiac rotation and wall motion from tethering segments.

The use of myocardial speckle tracking derived strain, and strain rate is based on software detecting speckles from within the myocardium, with 2D echocardiography analysing the motion. This technique has been used to assess the regional and global function of both the right and left ventricles with great success (Pirat et al., 2006) although more studies into RV function are needed to further evaluate the technique (Lindqvist et al., 2008).

Using an image-processing algorithm, the natural acoustic markers within the grey scale image are tracked throughout the cardiac cycle. The user creates a defined region of interest (ROI) by tracking along the endocardial border of the chamber in question. Irrespective of angle or chamber this ROI can be manipulated to fit both the endocardial and epicardial borders of the chamber.

Once fitted, the algorithm is designed to track individual speckles within blocks of 20 to 40 pixels, frame by frame. What makes this technique more versatile than TDI is that within the tracking algorithm, there is the ability to track the speckles in any direction overcoming many of the inherent problems encountered with TDI (Geyer et al., 2010). In addition to strain characteristics, displacement and velocity curves can also be displayed.

The speckles are assumed to be unique for each myocardial segment and thus provide a fingerprint from which the tracking can be made. This unique fingerprint must remain preserved throughout the cardiac cycle resulting in a high frame rate during acquisition (Teske et al., 2007).

The myocardial border is traditionally divided into six regions of interest for the RV and six for the LV. Throughout the cardiac cycle, a calculation of the global longitudinal strain and global longitudinal strain rate is displayed (Reisner et al., 2004). Because of the complex anatomy and the manner in which the RV contracts, measurement of RV deformation typically assesses the longitudinal function of the RV.

Conventionally utilised for assessment of the LV, STE has more recently been applied to the RV with promising results. Although cMRI is currently considered the gold standard and has been shown to correlate well with STE (Helle-Valle et al., 2005); (Amundsen et al., 2009) limitations due to portability and availability and restrictions due to metallic implantable devices and valves given the strong magnetic fields, means that there is a requirement for alternative imaging techniques with which to assess deformation characteristics (Horton et al., 2009).

At present functional assessment of the RV is almost exclusively limited to a visual assessment of wall thickening and area tracing, or measurements made at the basal position of the myocardium, used as a surrogate indicator of global function. To obtain accurate measurements in this fashion has been shown to require extensive training (Picano et al., 1991) but, even so these measurements remain subjective (Hoffmann et al., 1996)

Although both TAPSE, and pulse wave TDI measurements continue to be in common use, there remains a need for a functional tool that can account for changes in both the segmental and the global function of the RV. Similar to TDI, STE provides offline segmental analysis of myocardial velocities and deformation parameters whilst remaining angle independent, an important factor considering the complex nature of imaging the RV.

Studies that have calculated longitudinal RV strain data have previously used a combination of single (lateral RV freewall) wall tracking and dual (lateral and septal) to calculate longitudinal segmental strain data in a number of different patient groups (Tong et al., 2008, Meris et al., 2010, Fukuda et al., 2011, Teske

et al., 2009a). The prognostic efficacy of this single wall approach has shown a significant correlation with improved 6-minute walk tests in patients with PH and a reduced mean value prior to treatment compared to control volunteers (Fukuda et al., 2011).

This variation in acquisition technique could lead to alterations in normal segmental parameters given the effects of tagging between segments. The musculature of the shared septum is believed to be essential to the function of the RV freewall given the attachment via the moderator band and fibre orientation, (Buckberg, 2006) however the association between the LV and RV musculature is still unclear within the septum when assessed via ultrasound.

Despite the advantage of using STE to describe both LV and RV function, there still remains a lack of acknowledgement regarding its use in standard practice, with regards to normal values and the methods used for acquisition of results. In addition, assessment of both LV and RV diastolic function using STE remains limited due to a lack of normative data.

Changes in RV deformation characteristics have been identified with a number of different pathologies (Teske et al., 2009a) (Sachdev et al., 2011, Fukuda et al., 2011). Relatively little is known, however about what physiological parameters could influence both strain and strain rate, within the normal population.

A recent cMRI study into subclinical changes in LV function, conducted by the MESA group, found significant differences between ethnic groups. STE presents a new opportunity to assess early stage pathology where previous techniques for quantification have failed to identify significant changes. Ethnic variation in diastolic function has been identified with Hispanics and blacks showing worse diastolic indices than whites (Russo et al., 2010).

Spectral Doppler tricuspid forward flow is one method used to assess diastolic function with changes in E/A waves similar to that seen with the mitral valve with reduced compliance of RA filling. The use of RV diastolic functional assessment has been advocated within recent RV guidelines as a subclinical

marker of overall function with noticeable changes in diastolic function apparent before detectable systolic function (Rudski et al., 2010). The use of STE in the assessment of diastolic function not only reduces the load dependency issues faced by Doppler measurements and, to a lesser extent, TDI but provides the opportunity to assess the diastolic components of both regional and global areas of myocardium. This has the potential to further enhance the knowledge surrounding the development of diastolic heart failure with increasing age, whilst assessing the myocardial mechanics directly.

The notion that early and late diastolic phases change with age is well established using standard pulsed wave data, in particular for the LV (Nagueh et al., 2009). The assessment of RV diastolic function however, is limited (Rudski et al., 2010). STE provides a simple method of obtaining diastolic parameters with the addition of both regional and global function. As more work is undertaken to ascertain early subclinical changes in RV function, the use of speckle tracking derived diastolic parameters may help in identifying change in the pathological cascade of conditions such as pulmonary hypertension.

A recent study into the feasibility and reproducibility of RV strain measurements, using premature infants, was able to achieve good images in 84% of the study population after optimising the RV window (Levy et al., 2013). Despite the technical challenges faced with imaging infants and lack of compliance, this feasibility of 84% is unlikely to reflect an accurate representation of the adult population given the obvious anatomical limitations, increase in adipose tissue and the possibility of chronic lung conditions.

1.17. Right ventricular three dimensional assessment

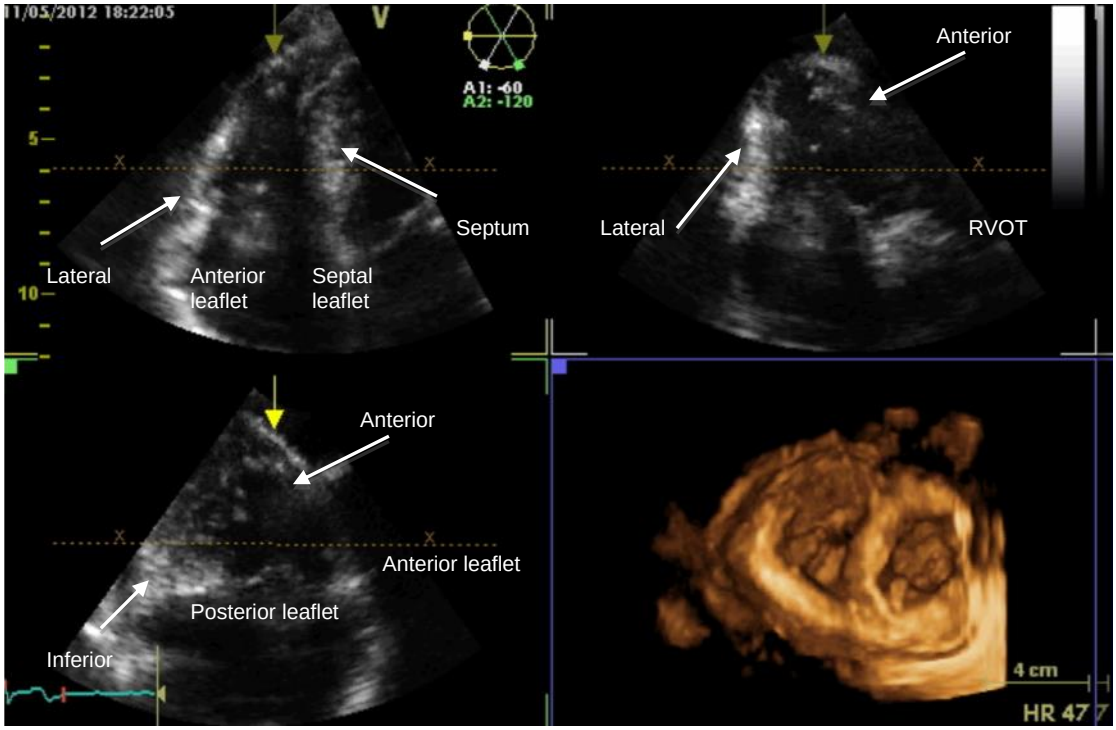


Figure 1.1 Transthoracic 3D assessment view acquired in the apical four chamber view using the 3D probe. The quad slice view allows assessment of each component of the RV in an alternative fashion to the standard 2D probe to allow the user to maximise endocardial definition. See section 1.17 for detailed explanation.

Accurate assessment of both RV size, via linear, area or volume measurements and RV functional assessment, is pivotal in the management of disease states known to directly or indirectly affect the RV. Real time 3D echocardiography opens up a new method of chamber and valvular assessment not traditionally possible due to geometric limitations with traditional echocardiography (Maffessanti et al., 2013).

Detailed analysis of both chamber size and function has advanced in recent years due to the ability to acquire three-dimensional data from both the left and right ventricle. Whilst early research was focused on the assessment of the LV, the opportunity to overcome many of the geometric and imaging limitations imposed by the RV, has seen a surge in 3D studies (Rudski et al., 2010) (Valsangiacomo Buechel and Mertens, 2012, Shimada et al., 2010).

Similar to STE, the use of 3D acquired cardiac data has been subject to testing against cMRI, the current gold standard and CT. These studies have shown that 3D volumes, EF and SV all compare favourably in both adults and children (Lu et al., 2008, Niemann et al.) (Gopal et al., 2007), and in patients with PH. (Grapsa et al., 2010) Despite good correlations between techniques, reproducibility for both EF and SV is higher with cMRI (Grapsa et al., 2010). 3D echocardiography displays less underestimation than standard 2D assessment (Gopal et al., 2007) and improved reproducibility when compared with cMRI (Jenkins et al., 2007). By comparison, other modalities such as CT have demonstrated an overestimation compared to cMRI of the RV carried out on the same group of patients (Sugeng et al., 2010). This underestimation has been deemed clinically significant, limiting the use of the technique within any group displaying more than mild RV dilation (Crean et al., 2011).

Calculations of RV volumes have traditionally been hampered by the complex anatomy of the RV and by changes in loading states, which can alter a subject's ejection fraction (Lang et al., 2005). A recent study demonstrated that in several different disease groups primarily affecting the RV, 3D echo was able to detect changes in RV modelling regardless of RV systolic pressure, due to pathological conditioning (Bossone et al., Grapsa et al., 2012). The increasing use of 3D data comes as a result of the poor volumetric assessment of the RV made using standard 2D methods (Lai et al., 2008).

The introduction of measurement software designed to analyse and create a model of the RV using 3D ultrasound (figure 1.1), as well as calculating RV volume and assessing function is an attempt to standardise and minimise these problems.

Work conducted by (Niemann et al., 2007) validated the use of the RV TomTec analysis model by comparing results of 3D volume (ml) calculations using offline 3D analyses and cMRI in healthy adult subjects (n= 14) and a group of children with congenital heart disease (n = 16). The results showed that the 3D echo RVEDV and RVESV volume data was accurately calculated when

compared to cMRI images in both groups (correlation coefficients r = 0.99 and r = 0.98 respectively).

This is due in part to the acquisition of the same cardiac cycle throughout the 3D measurement. Multiple views are displayed simultaneously; and a trace made of the endocardial border in one view that is then applied to each of the subsequent views. Unlike previous studies that have noted limitations to their work due to anatomical difficulties, in particular identifying the trabeculations and the RV moderator band, recent studies (Niemann et al., 2007) found these to be challenging but not statistically significant with reassuring measurements for both intraobserver and interobserver variability.

They conclude, by suggesting that a set of reference values should be obtained for RV volume and function in the normal population (Niemann et al., 2007), a need that was also highlighted in the ASE 2010 guidelines (Rudski et al., 2010).

Studies have shown that gender, age (Tamborini et al., 2010), and ethnic differences have been found whilst studying 3D data within healthy populations, using both 3D echocardiography (Chahal et al., 2012b) and cMRI (Natori, 2006). Gender differences have since been investigated in a recent study that imaged 507 healthy volunteers across many age ranges in an attempt to derive normal 3DRV models (Maffessanti et al., 2013).

The results of this study demonstrated that much like standard two dimensional measurements (Willis et al., 2012), 3D RV volumes also demonstrated gender, age and body size differences within this homogenic group of healthy volunteers (Maffessanti et al., 2013).

Interestingly the volunteers within this group contained a number (*n* 245) who had been previously reported under another study (Tamborini et al, 2010) three years previously, also investigating healthy volunteers and showing a similar trend with gender age and body size (Tamborini et al., 2010). This well-designed study, which utilises the collective experience of several large centres,

acknowledges the homogeneity of the study sample which therefore fails to account for ethnic variation.

Despite the lack of cMRI within this study, there is sufficient evidence to suggest that the two techniques differ enough that they should be considered as two separate diagnostics tests with a consistent underestimation of RV volumes when compared to cMRI (Shimada et al., Maffessanti et al., 2013) resulting in two sets of reference values.

In addition to defining simple and ratiometric measurements of RV volumes, (Maffessanti et al., 2013) also utilised allometric scaling of RV volumes to effectively remove the influence of BSA giving true size independent measurements. They demonstrated a positive correlation with body size for EDV ($r=0.55$) ESV ($r=0.45$) and SV ($r=0.50$) ($p<0.01$ respectively) but a reduction with aging (EDV $r=-0.36$; ESV $r=-0.37$; and SV $r=-0.25$; $p<0.01$). Maffessanti and colleagues systematically assessed each variable within their analysis however the application of these modelled formula poses a greater challenge within typical clinical settings given their complex nature.

1.17.1. TomTec

The use of 3D volume analysis requires considerable post-processing of the acquired dataset. This has been available on a number of manufacturer platforms, based on the LV. However, the introduction of a dedicated RV software program (TomTec 4D-RV Analysis, Unterschleissheim, Germany) has allowed less reliance on geometric assumptions and modelling. The forth dimension (4D) referred to in the software title, refers to the ability of the software to display the movement of the 3D dataset throughout time i.e. the systolic and diastolic components of the cardiac cycle.

The TomTec program involves semiautomatic quantitative analysis of the 3D dataset throughout the cardiac cycle. The result is the calculation of end diastolic

volume (EDV) and end systolic volume (ESV) in ml. From this both EF and stroke volume (SV) are calculated.

The software has been validated in a number of studies using cMRI (Niemann et al., 2007, Grewal et al., 2010, van der Zwaan et al., 2010, Leibundgut et al., 2010) and against radionuclide techniques (Kjaergaard et al., 2006a) which proved less favourable than later studies (Kjaergaard et al., 2006a). In addition, the use of contrast agents to improve the opacity of the endocardial borders was subsequently reported to then reduce the leaflet definition which remains key to post-processing (Kjaergaard et al., 2006a).

This platform is vendor input independent therefore allowing comparable studies across different manufacturers, leaving only variations in acquisitions settings as a possible source of error within each study (Maffessanti et al., 2013). Traditionally these studies have involved patients with either congenital abnormalities or impaired RV function, however given the challenges faced with the prospect of multiple cMRI in healthy individuals this is to be expected.

Acquisition of 3D images requires a full matrix array transducer to produce the 'block' of 3D data. This data is then transcribed by the TomTec software into three orthogonal views from which semi automated readings are made. A detailed description is available within section 2.12. These views provide the unique feature previously missing with RV assessment and allow visualisation of all three components of the RV anatomy, relative to one another.

The data-set is still subject to the same limitations of echocardiography as a technique, however, with 3D data studies displaying limited temporal resolution (Perrin et al., 2012). These reductions in temporal resolution may be key in endocardial definition of the true end-systolic frame which by virtue of the calculation can affect RV EF (Tamborini et al., 2010).

1.18. Reproducibility

To ensure that these techniques both traditional and more advanced are applicable for clinical use, the reproducibility of all the techniques used within the study must be described and reported. Results can be reported in a number of ways with the most basic, (best case scenario) being the assessment of the same image by the same observer on separate occasions. This is reported as the intraobserver analysis. Further to this a second observer can be introduced to measure the same image (blinded to the first observer's results). This is reported as the interobserver analysis.

What this form of testing shows is the amount of variability derived between the interpretation of the same image by two observers, and is often reported in a number of ways, one being agreement via Bland Altman agreement reported as a 95% limit of agreement statistic (LOA). This variability may vary due to skill levels, measurement timing or interpretation of, for example, the myocardial borders. All of which are common variables that can be encountered when the technique in question is applied with clinical meaning.

As with any technique, the reproducibility of both the dimensions and functional assessment is a vital component that must be established (van der Zwaan et al., 2011b). This can be undertaken at a number of levels, from the best-case scenario – intraobserver through to test–retest analysis, a component often overlooked and yet common within clinical practice when assessing patients over a number of years.

With prospective ultrasound studies some element of measurement variability or reliability should be anticipated. Many studies have undertaken the task of reporting interobserver and intraobserver analysis, but few have assessed the impact that image acquisition can have on the measurements recorded. Unlike the LV which has several anatomical makers available to pinpoint the correct plane in which to image, there remains conflicting advice regarding imaging of

the RV. In addition, the use of 3D assessment requires suitable experience to ensure that the images are being acquired in a suitable plane across all dimensions.

Bland and Altman identified the need for assessing measurement variation between methods of similar assessment, in their case, in peak flow meter readings (Bland and Altman, 1999) but the notion of agreement remains within studies aiming to define reference ranges for a clinical group. Often quoted are several different measurements. These include the LOA, coefficient of variation (COV), and intra class correlation coefficient (ICC).

Bland and Altman correctly identified that a simple bivariate correlation between two techniques measuring the same property should result in a close correlation, however this itself does not promote agreement between the techniques (Bland and Altman, 1999). Therefore as noted above, additional testing is required to establish the agreement but also the repeatability and the reliability of the measurements. LOA, COV and ICC scores have all been used within RV studies, across linear calliper, area, STE and 3D volumes measurements (van der Zwaan et al., 2011b, Oxborough et al., 2012a, Maffessanti et al., 2013, Willis et al., 2012).

Individually these techniques are designed to assess how comprehensive measurements are when tested across a number of scenarios, which typically include comparisons between users, and across separate acquisitions.

2. General Methods

The methods described in this chapter are common to all three study components within this thesis. Where specific techniques have been used to enhance the analysis, these have been described in greater detail.

2.1. Preliminary Information

Ethical approval was applied for at each of the three centres taking part within the research Full approval was granted by each centre (Appendix 7.1 – 7.3). All participants within the study were volunteers. Each volunteers received an information sheet and consent form prior to the collection of any data. A physical exam was conducted by a consultant or specialist registrar in cardiology, as well as a formal consultation where information relating to the volunteers health and medical status was recorded. Basic anthropometric data was recorded on all participants. Resting blood pressure (mmHg), height (m/cm) and body mass (kg) was used to calculate body surface area (BSA) and body mass index (BMI). Calculation of BSA was undertaken using the Dubois Dubois formula (equation 2.0) (Du Bois D Fau - Du Bois and Du Bois, 1916).

Equation 2.0 The Du Bois Du Bois formula for the calculation of BSA in m^2

$$BSA = 0.007184 \times W^{0.425} \times H^{0.725},$$

Where *W* is weight in kg and *H* is height in cm.

BMI was calculated using the Quetelet formula shown in equation 2.1 (Eknoyan, 2008).

Equation 2.1 The Quetelet formula for the calculation of BMI in kg/m^2

$$BMI = mass/ (height)^2,$$

Where mass is the body mass in kg and height is measured in meters.

Each volunteer underwent a 12-lead Electrocardiogram (ECG), used to ascertain if any rhythm or conduction abnormalities were present. This information was used in conjunction with the physical examination and health and lifestyle consultation to establish the suitability of volunteers for this study.

As specified by the ethics committee, any volunteers who were found to have undiagnosed cardiac conditions prior to or during the investigations were either referred to their General Practitioner or placed under the care of the respective cardiac consultant. Exclusion criteria can be seen in Table 2.0. In addition, where any structural functional abnormalities were diagnosed on the Echocardiogram, the volunteer would be excluded from taking any further part in the study. High level athletic activity was defined as >8 hours per week of endurance type activity (Teske et al., 2009b).

Table 2.0 Study exclusion criteria

Hypertension
Diabetes
History of thromboembolic disease
High level athletic competition ≥8 hrs/ week
Asthma /Chronic Obstructive Pulmonary Disease (COPD)
Obstructive Sleep Apnoea
Collagen Vascular disease
LV systolic dysfunction
Aortic/Mitral valve disease
Congenital Heart Disease
Human Immunodeficiency Virus
Portal Hypertension,
History of anorexigen usage

2.2. Sample Size Calculation

Sample size calculation was based on data provided by previous research within each of the three fields investigated. The sample size required was determined using the following formula.

Equation 2.2 Sample size calculation used to determine the size of the group for each assessed faculty.

$$N = 1 + (2(Z\alpha + Z\beta^2 SD^2)/(X_1 - X_2)^2),$$

Where Zα is the Normal Score for the level of significance α (set to 0.05 with a Normal Score of 1.96); Zβ is the Normal Score for the power required, β. Here β has been set to be 0.8 giving a Normal score of 0.8416. X^1 and X^2 are mean sample values for each of the respective methods derived from the literature with the standard deviations (SD) reported in Table 2.1.

The minimum detectable difference (MDD) was set at a level determined to be clinically relevant for each measure. This is shown in Table 2.1 Sample size was based on a primary (1 x 5) analysis of ethnicity, with a secondary analysis of gender. Standard deviations were taken from results derived from previous research (Foale et al., 1986, Teske et al., 2008, Tamborini et al., 2010) with an agreed MDD.

Table 2.1 Calculated sample size.

	SD	MDD	n
2D Calliper (mm)	0.50	0.50	17
Strain (%)	7	5.00	32
Strain Rate1-s	0.50	0.50	17
3D Volume (ml)	21	15	32

Using an alpha value of alpha = 0.05 and beta = 0.80 a minimum n value of 32 was suggested per ethnic group equating to a total group size of 175 volunteers. To account for possible poor image quality post analysis, additional volunteers were recruited to each group aiming for a minimum of 40. This would account for similar drop out in numbers encountered with other large studies using speckle tracking analysis (Dalen et al., 2010). Each group would consist of approximately equal number of male and female volunteers. Secondary analysis regarding gender would be conducted on the total sample size. Efforts were also

made to obtain a wide age spread to account for potential changes due to increasing age.

2.3.Echocardiography

The process and literature surrounding echocardiography has been detailed in section 1.13. Here specifics pertaining to each technique and the measurements made are described in greater detail.

Each volunteer received a full assessment of chambers, valves function and dimensions for both the left and right side of the heart. Standard windows were utilized to maintain consistency throughout. All images were acquired by one of five experienced sonographers and trained in the specific protocol for this study. All images were acquired using a systematic approach via a pre defined study protocol in order to maintain

Two identical commercially available ultrasound systems (Vivid 7, GE Medical Systems, Horten, Norway) were used to acquire all the images. Both system had two ultrasound probes attached, the same commercially available 2D 1.6 – 4 MHz phased array transducer and a 1.7 – 3.5 MHz phased array 3D transducer for the acquisition of both 2D and 3D images respectively. To ensure the study settings remained the same between centres, a protocol was designed and installed on both machines that maintained the same frequency, depth and cycle count as standard between studies and across the three centres.

2.4.Recruitment

Identification of the ethnic groups from both Malaysia and the UK that represented the largest national demographic population was the first stage in listing potential candidates. This information was based on the then current

respective national census data (Dobbs et al., 2006, Department of Statistics, 2000) which identified European, Malay, Chinese and Indian groups.

With the inclusion of Chinese volunteers from the Malaysian cohort, and given the extensive prior research demonstrating ethnic differences featuring this particular group, Afro-Caribbean volunteers were also recruited from a UK site.

The European sample was recruited via the Royal United Hospital, Bath UK. Chinese, Malay and Indian volunteers were recruited via the Gleneagles Medical Centre, Pennang, whilst Afro-Caribbean volunteers were recruited via the Royal Free Hospital in London.

The recruitment process was conducted using a number of sources. Advertisements were placed around the respective local area, within the hospitals, with universities, councils and community groups. Volunteers were initially asked to contact via email or face-to-face, where they would receive a pre-study information pack. Once contact had been made, face-to-face or phone interviews were conducted to ascertain any potential exclusion criteria to the study prior to being consented. This delay ensured that all participants had sufficient time to read and make an informed decision about their involvement.

In order to allow a free expression of ethnicity and ethnic heritage, information pertaining to subject ethnicity was discussed with volunteers through a combination of both self-assignment and examination regarding their maternal and paternal heritage during consent and selection. This hybrid method was believed to address some of the issues raised in section 1.5 regarding a more single-handed approach to the use of ethnic groups. Importantly, the participants felt appropriately represented and consented to their involvement.

2.5. Image Acquisition

Echocardiograms were performed in the left lateral position. All scans were conducted using a standardised protocol devised for this study in conjunction with both ASE guidelines (Lang et al., 2005) (Rudski et al., 2010) and previous work (Foale et al., 1986). This protocol is available in the appendix section 7.4.

All images were digitally stored for offline analysis using specific post analysis software (EchoPAC Version 8.0, GE, + TomTec, Germany). 2D images were acquired using harmonic imaging. All images were optimised within the device parameters to maximise spatial and temporal resolution. Each was assessed for optimal image quality and adjusted as required using gain, dynamic range, depth, angle width, frame rate and frequency. Specific techniques required adjustment to the frame rate. This was achieved by narrowing the sector width and manually increasing the frame rate.

Volunteers began the scan in the left decubitus positions with the bed angled to approximately 45° and the left arm raised to enhance the inter-thoracic space. Example images recorded from each of the windows used can be seen in figures 2.0 to 2.12 with additional anatomical descriptions.

In each window, foreshortening of the image was avoided (Lang et al., 2005). To ensure adequate orientation and level of the RV, in addition to anatomical markers such as the moderator band and delineation of the apex, the left and right atria were used.

In the instance of a suboptimal image, a second sonographer would attempt to acquire the image. In addition to moving the position of the volunteer, respiration was controlled so that minimal lung field would influence the image. Studies that resulted in no measureable data due to poor image quality were removed from the analysis.

2.6. Repeatability

We sought to establish the repeatability of each diagnostic test by employing a test – retest process. Reproducibility for each linear, strain and 3D measurement was assessed in a subset of 40 volunteers chosen at random. An accredited reader experienced in echocardiographic analysis undertook all initial measurements in the cohort and then re-measured the same images at least two weeks after the first analysis, blinded to the initial results. This was used to establish the intraobserver (reader variability) variability for each measurement. The same subset was then evaluated by a second accredited reader, blinded to the results of previous analysis, to assess the interobserver variability (reader vs. reader variability).

In addition to the primary acquisition, 30 volunteers then underwent a second scan performed by a second sonographer. Analysis of these additional images was performed by reader 1 and was used to calculate test-retest variability (acquisition vs. acquisition variability).

For each testing parameter, COV, ICC, and agreement using Bland and Altman's LOA analysis was calculated. COV was calculated using the standard deviation of the difference between the two measurements, multiplied by 100 and divided by the mean value, calculated as a percentage (Tamborini et al., 2010).

Utilizing a method for the assessment of ICC previously reported (Calleja et al.) the following guidelines suggested by Shrout and Fleiss (Shrout and Fleiss, 1979) were implemented. ICC >0.75 = excellent, 0.4 to 0.75 = good, and <0.40 = poor. 95% limits of agreement (LOA) were calculated from 2 standard deviations of the mean using methods described by (Bland and Altman, 2010).

Results were calculated using Graphpad Prism version 6.0. These were plotted on a scatter graph used to illustrate the level of agreement between the two methods. This method comparison methodology was employed at each of the three test levels. Acceptable levels of agreement were pre-determined based on previous research and clinical experience.

2.7. 2D Linear measurements

Measurements were made using those suggested by Foale et al (1986) and ASE/EAE guidelines for both reference and comparison (Lang et al., 2005, Foale et al., 1986). Table 2.2 lists the location of each measurement shown in Figure 2.0. Volunteers received a full quantitative assessment of LV size and function to ensure that no LV systolic dysfunction, significant diastolic dysfunction or valvular abnormalities existed.

Comparisons were made between several major papers in the assessment of RV size using 2D calliper measurements. Using the then current guidelines from both the ASE and EAE devised by Lang et al (2005) the location and description of each measurement of the RV was scrutinized (Lang et al., 2005).

Ten measurements were identified for the assessment of RV size. These were conducted in the parasternal long axis to assess RVOT (RV Outflow Tract) width and wall thickness (RV WT), parasternal short axis (at aortic valve level) to assess the RVOT and apical four chamber view to measure the RV inflow and apex. Images were angled to maintain a consistent view of both the RV lateral wall and septum with optimization of the apex to give clear delineation.

Measurements were made at end diastole, identified using the widest tricuspid valve (TV) leaflet excursion prior to the onset of the ventricular depolarisation represented by the QRS complex (Lang et al., 2005). All cavity measurements were made inside edge to inside edge.

Measurement of the minor axis (RVD-1) was made within the basal third of the RV below the tricuspid valve at the widest portion with RVD-2 measured at the mid cavity level (Foale et al., 1986). In addition, an annular measurement, RVD-AN (see Table 2.2) was also taken and used as a reference point for the calculation of RVD-3.

RV function was assessed using Tricuspid Annular Systolic Plane Excursion (normal TAPSE ≥1.6cm,), RV fractional area of change (FAC) (normal >35%) and pulsed Doppler peak velocity at the annulus (normal RV TDI ≥10 cm/sec) as defined within (Rudski et al., 2010). Images were optimised using both sector width and focus position. Images were all taken at end expiration to minimise translational movement and lung artefact.

Images were initially considered of adequate quality if each of the associated valves could be visualised throughout the parasternal and apical views, and the major parts of the cavities could be seen (Foale et al., 1986). Each picture was optimized to ensure that the relevant anatomy was available for measurement and at the best available orientation while maintaining the appropriate plane in which to measure the widest dimension.

Table 2.2 Location of RV 2D linear and area measurements

Measurement	Location of measurement
RVD-AN	The hinge point attachment of septal leaflet to septal wall and anterior leaflet to lateral wall. (Anwar et al., 2007, Foale et al., 1986)
RVD-1	Taken within one third of the distance below the tricuspid valve annulus towards the RV apex. (Foale et al., 1986)
RVD-2	Mid right ventricular diameter measured at the mid cavity level as described by Foale et al (1986)
RVD-3	Mid point of RVD-AN in the major axis to the endocardial boarder of the RV apex(Foale et al., 1986)
RVOT-1	Perpendicular to the central point of Aortic Valve closure line to the endocardial border. Measurement made at peak of the R wave (Foale et al., 1986)
RVOT-2	Measurement made just below the Pulmonary Valve annulus, inner border to inner border. (Foale et al., 1986)
RVOT-3	Proximal region of the RVOT in PLAX view. Interventricular septum to anterior RV free wall (Foale et al., 1986)
RV WT	M-Mode of the RV free wall in the PLAX view (Foale et al., 1986).
RVESA	Endocardial border traced from apical four chamber view at the time of the smallest RV cavity(Lang et al., 2005)
RVEDA	Endocardial border traced from the apical 4 chamber view at end diastole(Lang et al., 2005).

Measurements adapted from the work of Foale et al (1986), Anwar et al (2007) and Lang et al (2005).

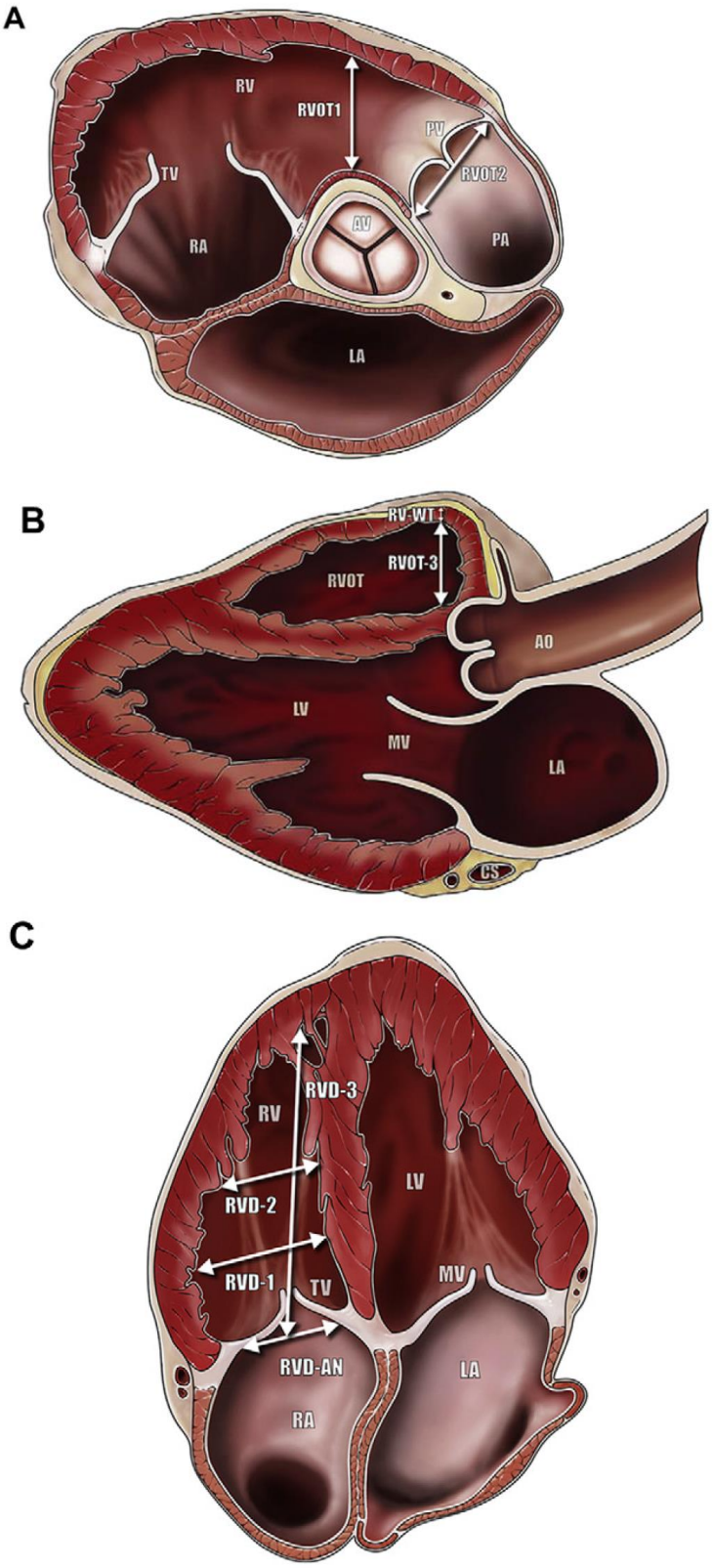


Figure 2.0 Sample images showing measurements of the RV at end diastole. (A) Sample images representing views and measurements from the parasternal short axis view at the aortic valve level showing RVOT-1 and RVOT-2. (B) Parasternal long axis view showing RVOT-3 and RV WT. (C) Apical four chamber view. Within this view, assessment in the major and minor axis is possible. RVESA and RVEDA are also made from this view.

2.8. Standard 2D Echocardiography

The following are standard echocardiographic views with anatomical descriptions. These have been referenced from the anatomical examination of the normal right heart undertaken by Ho and Nihoyannopoulos (2006). Figure 2.0 displays the location of each of the linear measurements with the RV and RVOT. Full descriptions of these measurements can be found in Table 2.2.

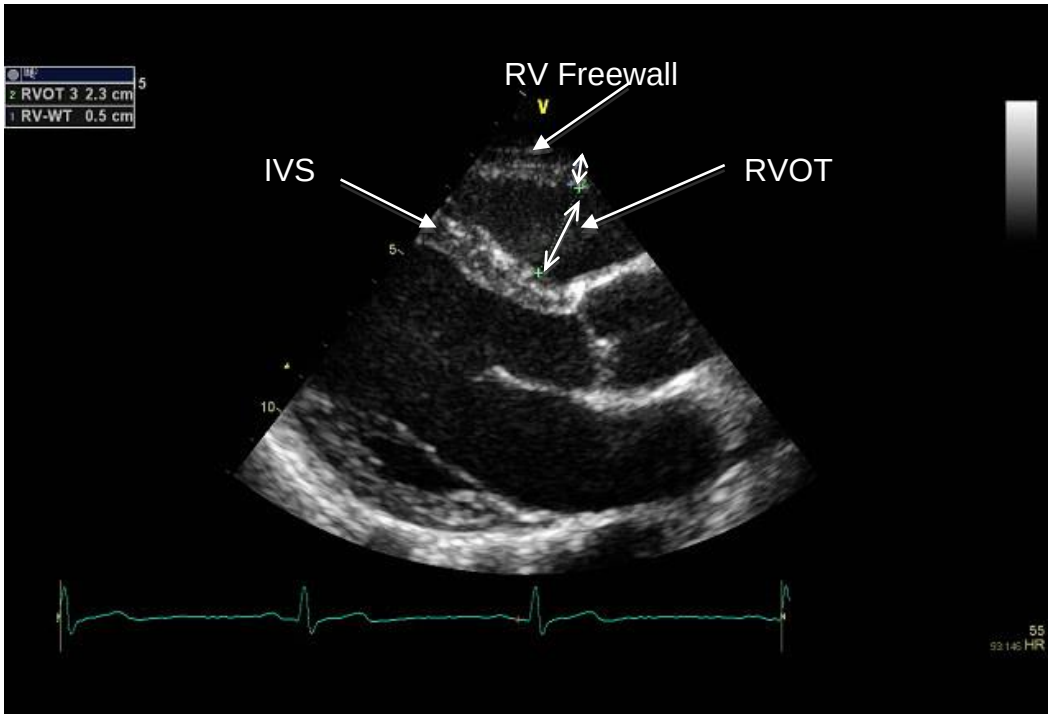


Figure 2.1 Parasternal long axis plane displaying both RVOT-3 and RV wall thickness measurements

Taken adjacent to the left of the sternum, within the intercostal space, the PLAX view (figure 2.1) displays both the RVOT and RV freewall. Posteriorly to the RV freewall, the interventricular septum (IVS) is seen which, via the moderator band links the IVS and RV freewall. RVOT-3 and RV-WT measurements are made in this view (Ho and Nihoyannopoulos, 2006).

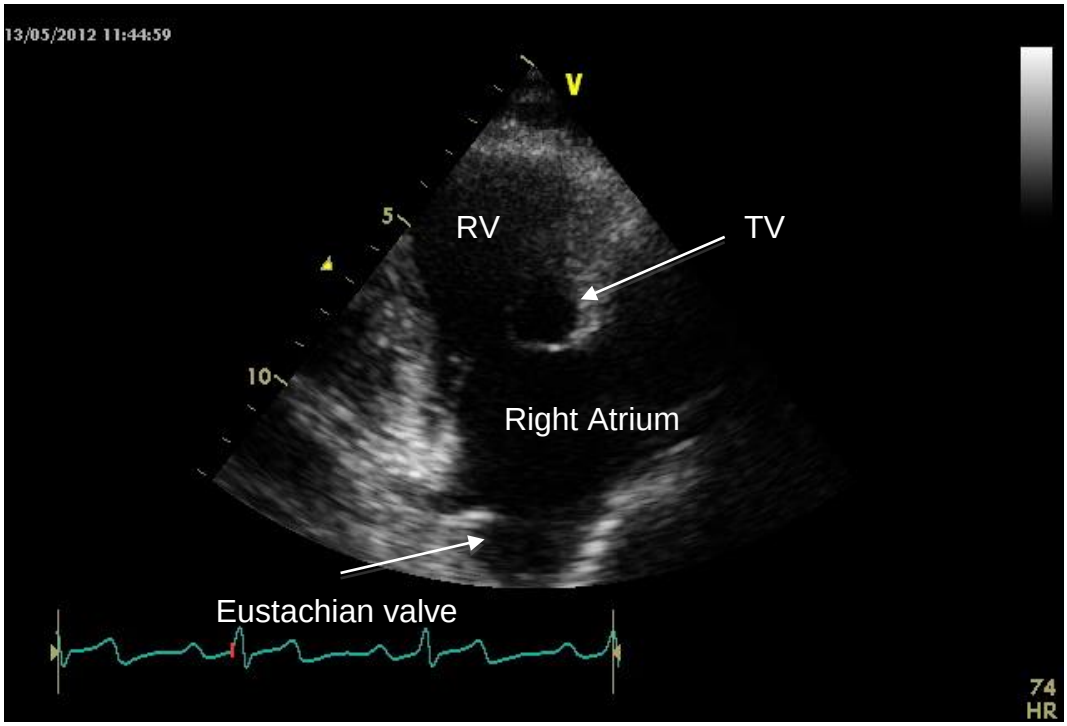


Figure 2.2 Right ventricular inflow tract displaying the antero-superior and mural leaflets of the TV.

With the transducer beam angled towards the xiphoid from the parasternal long axis view, the beam traverses the inflow of the RV (shown in figure 2.2), displaying the tricuspid valve and annulus. The RV is featured displaying more of a spade shape. Here the Eustachian valve can be seen. Colour flow is used to assess the TV for any regurgitation. If apparent, a TR envelope can be acquired and measured using continuous wave (CW) Doppler (Ho and Nihoyannopoulos, 2006)

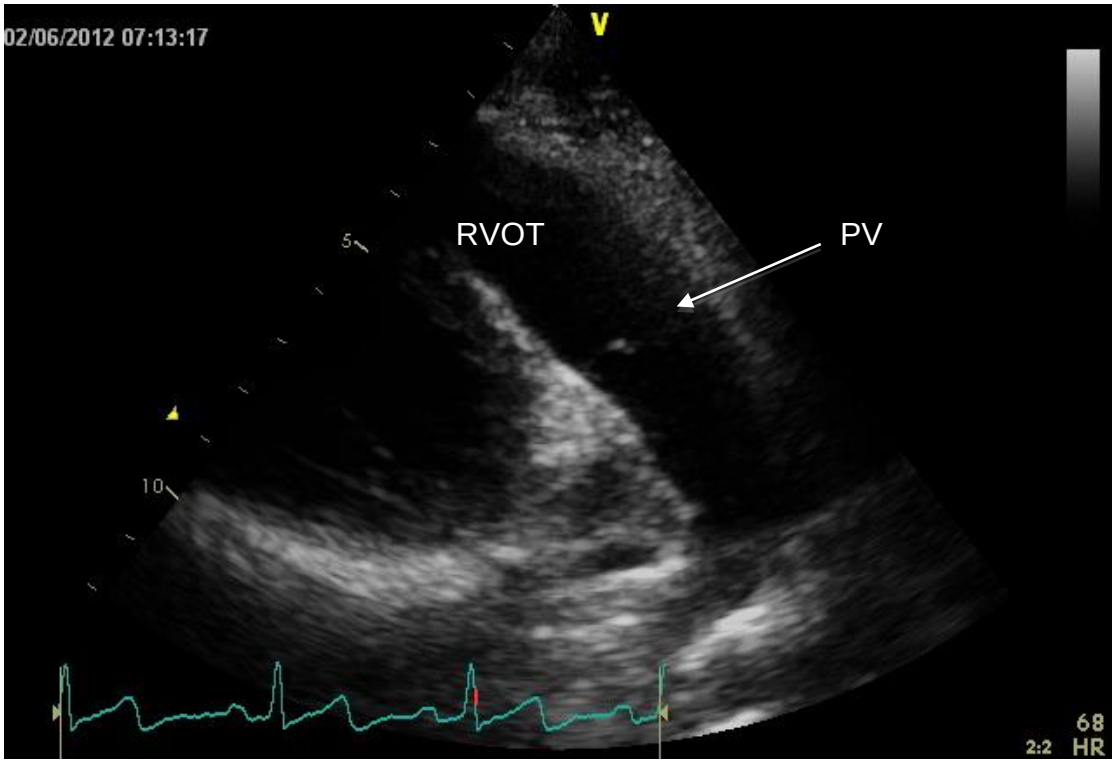


Figure 2.3 Right ventricular outflow tract displaying the PV.

From the same horizontal plane the transducer beam can be angled towards the left shoulder to display the proximal outflow portion of the RV (see Figure 2.3). Here two of the three pulmonary valve leaflets can be seen and assessed. Using figures 2.2 and 2.3 both the inlet and outflow tract, or infundibulum, have been imaged (Ho and Nihoyannopoulos, 2006).

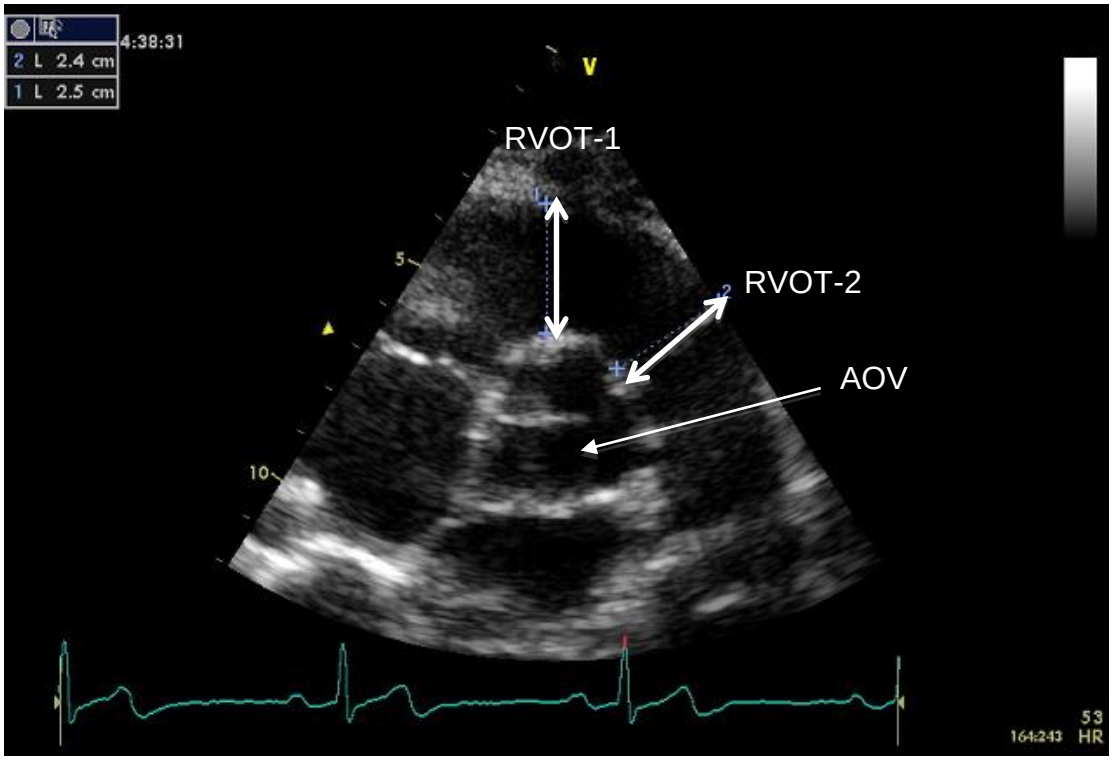


Figure 2.4 Parasternal short axis view assessed at the aortic valve level

Figure 2.4 displays the parasternal short axis view (PSAX), which demonstrates the crescent shape of the RV as it curves around the LV allowing visualisation of both the inflow and outflow valves (Ho and Nihoyannopoulos, 2006). The aortic valve provides a convenient anatomical marker from which reference measurements can be made.

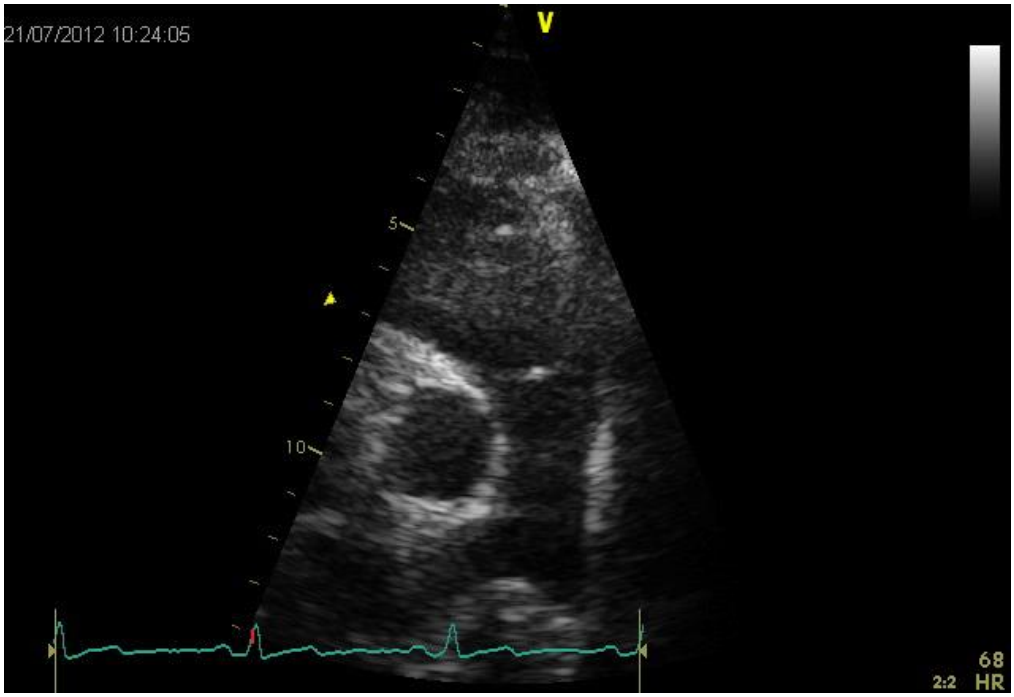


Figure 2.5 Pulmonary valve bifurcation view, optimised from a modified parasternal short axis view.

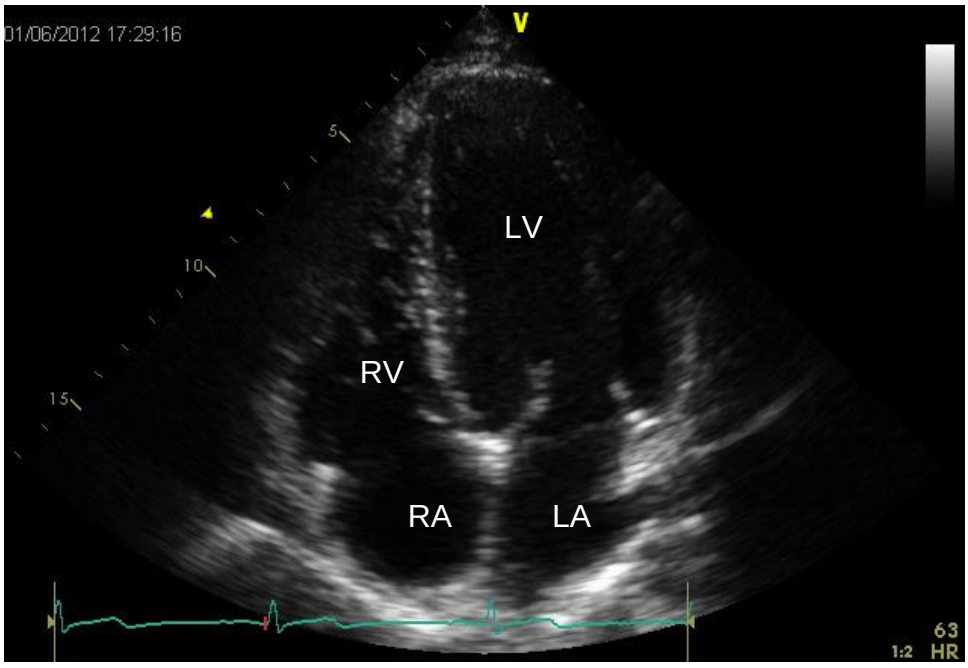


Figure 2.6 Apical four chamber view taken from the apical window that lies in a more lateral position

Here calliper measurements perpendicular to the aortic valve and at the level of the pulmonary valve can be acquired. A clearer view of the pulmonary valve can be obtained by optimising the sector width and tilt of the image to allow

visualisation of the pulmonary artery and bifurcation of the pulmonary trunk as seen in Figure 2.5.

The apical four chamber view shown in Figure 2.6, provides the most complete view from which to assess the all four chambers. Here morphological assessment can be made as well as measurements of the tricuspid valve annulus in conjunction with other RV dimensions (Ho and Nihoyannopoulos, 2006). Optimisation of this view remains important to ensure measurement of the maximum RV dimension (Rudski et al., 2010).

This view is, however hampered by the dense trabeculations that can often be seen limiting the near field apex. Careful optimisation of the focus point and gain settings can help to minimise this. Identification of the moderator band, made within this view, remains a defining characteristic of the right ventricle (Ho and Nihoyannopoulos, 2006).

Using a focused RV view, shown in Figure 2.7 from the apical orientation, measurements of the major (RVD-3) and minor axis (RVD-AN, RVD 1-2) can be made at end diastole defined by the last frame prior to tricuspid valve closure (Willis et al., 2012). Functional measures of RV contractility and area can also be made within this view. Right ventricular fractional area of change (RVFAC) is obtained by tracing the endocardial border in both end diastole and in end systole as previously defined (see figure 2.8).

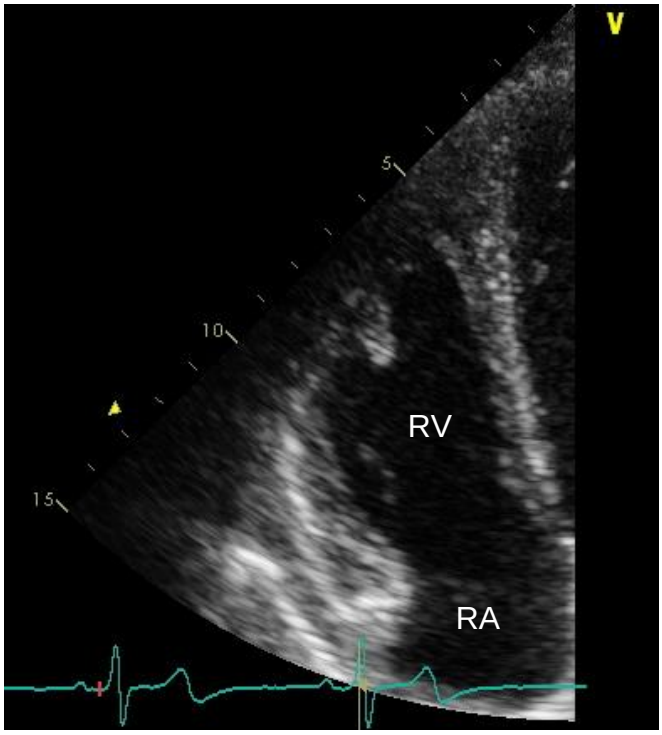


Figure 2.7 Focused RV view optimised for RV assessment from the apical four chamber view and the inflow components.

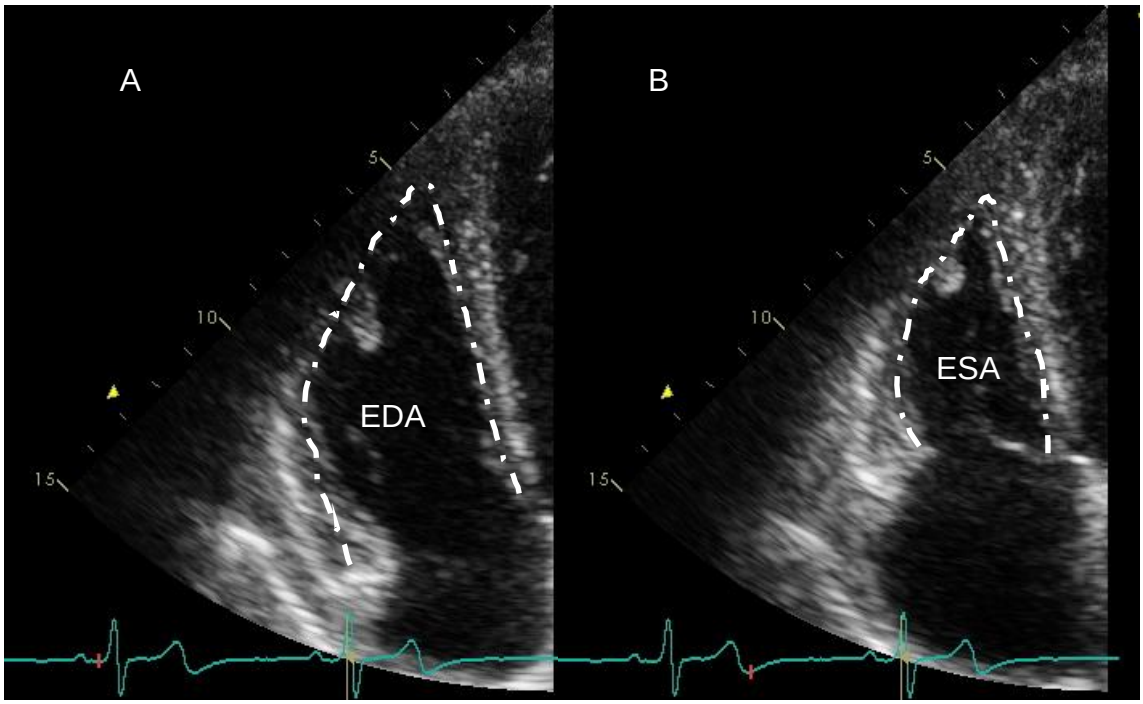


Figure 2.8 (A) RV end diastolic area trace and (B) RV end systolic area trace demonstrating the endocardial border tracing.

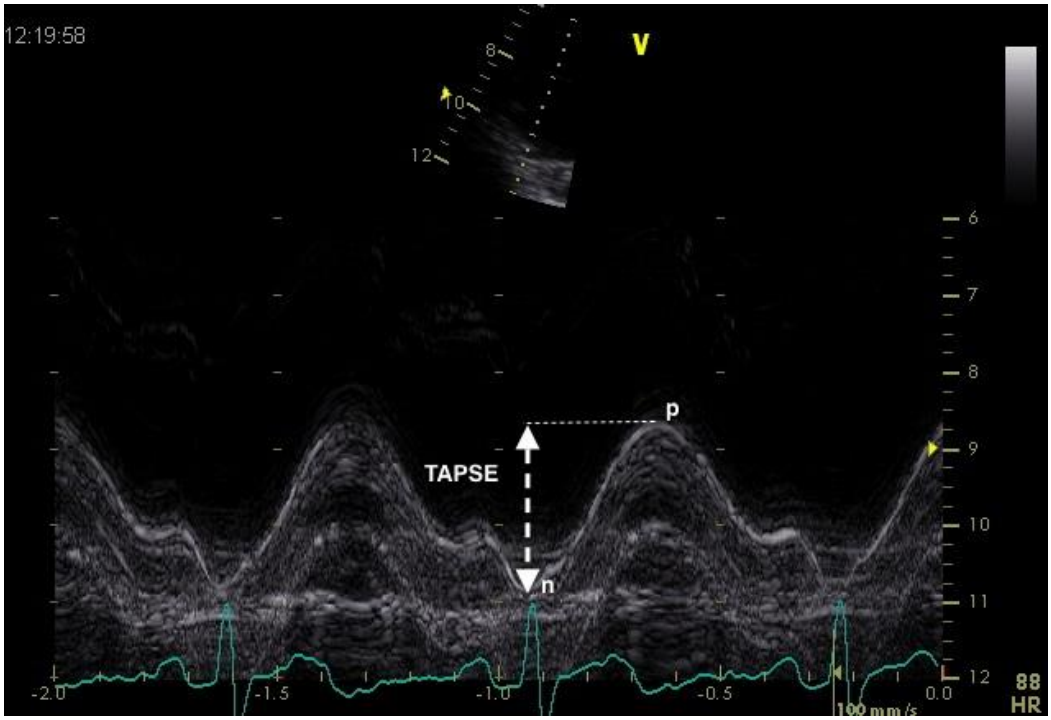


Figure 2.9 RV M-Mode measurement of the TV annulus systolic excursion (TAPSE)

Measures of function, TAPSE and 'S' prime are made from these views using M-mode and TDI respectively (see Figures 2.9 and 2.10). TAPSE was defined as being measured from the nadir (n) to the peak (p) in the junction between the RV freewall and the TV annulus in the A4C view (Rimington and Chambers, 2007). A sweep speed of 50 to 100 mm/s was used to record all M-mode and PW-TDI images.

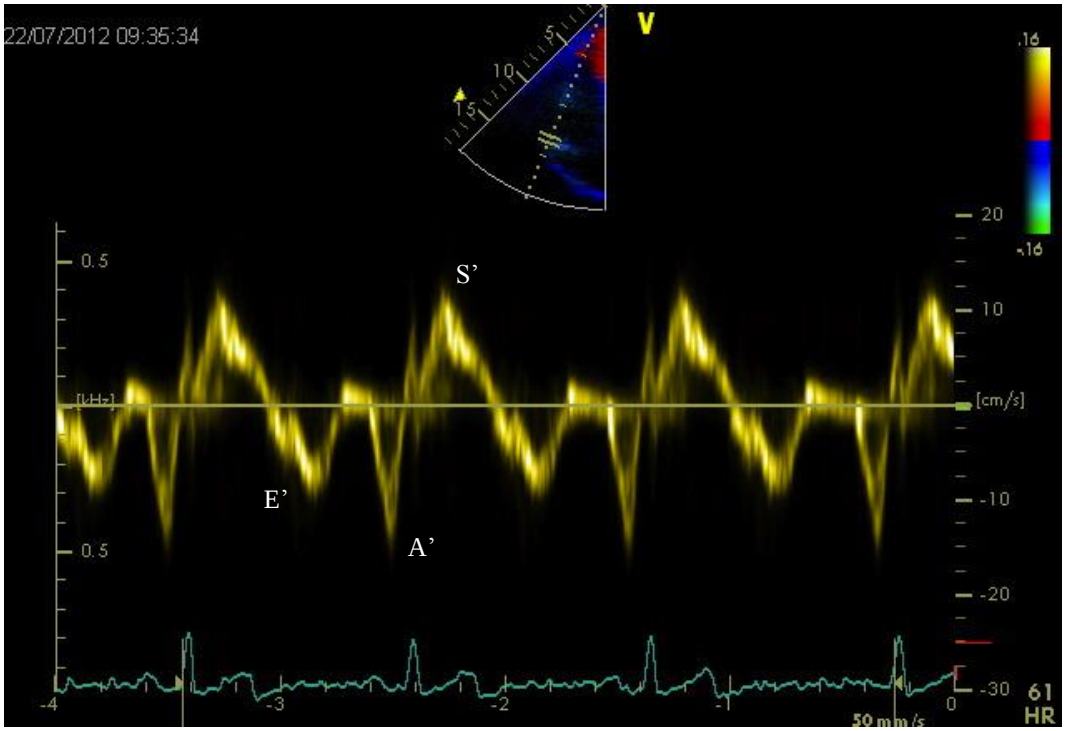


Figure 2.10 RV TDI PW Doppler of the TV annulus

From the apical four chamber view, anterior tilting of the transducer opens the LVOT and proximal ascending aorta into the apical five chamber view and allows CW Doppler of the aortic valve and PW Doppler of the left ventricular outflow tract (Ho and Nihoyannopoulos, 2006).

A 90° anticlockwise rotation from the apical four chamber view brings in the apical three (long axis) chamber view (see Figure 2.11). A portion of the RVOT can be seen here again tapering off towards the mid portion of the septum. 30° of counter clockwise rotation present the apical two chamber view in which no RV components are displayed (see Figure 2.12) (Ho and Nihoyannopoulos, 2006).

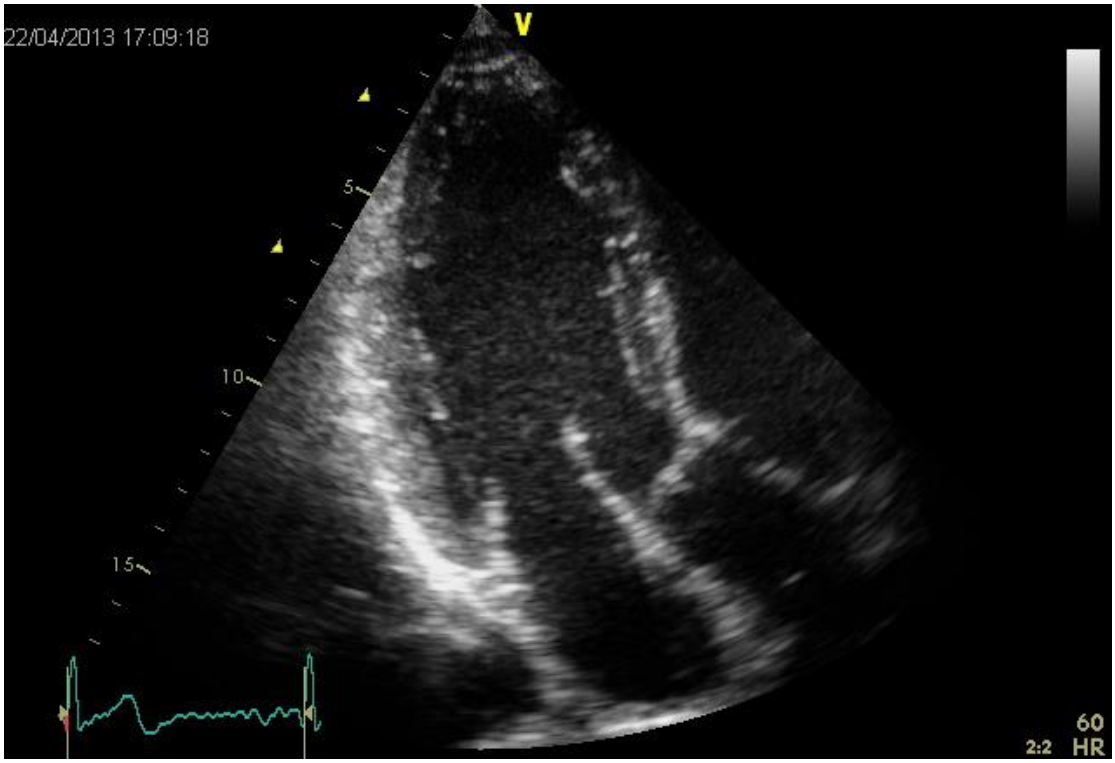


Figure 2.11 Apical Three Chamber View.

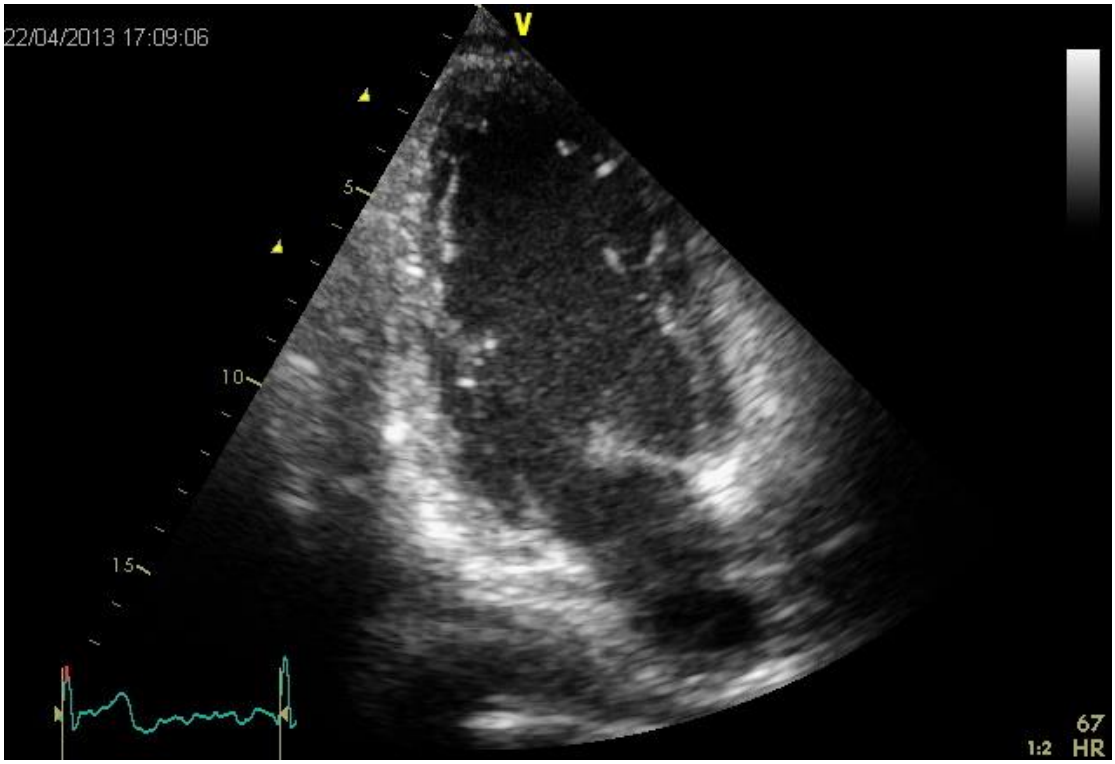


Figure 2.12 Apical Two Chamber View

2.8.1. Standard Doppler imaging

PW Doppler signals measured at both mitral and tricuspid inflow was used to assess diastolic function. The PW signal was obtained in the A4C view by placing the 4mm sample curser at the tips of the MV and TV respectively. The spectral signal (see Figure 2.13) was optimized for signal to noise ratio using the low velocity reject and baseline control. This allowed measurement of the passive (e wave) and active (a wave) filling into both ventricles.

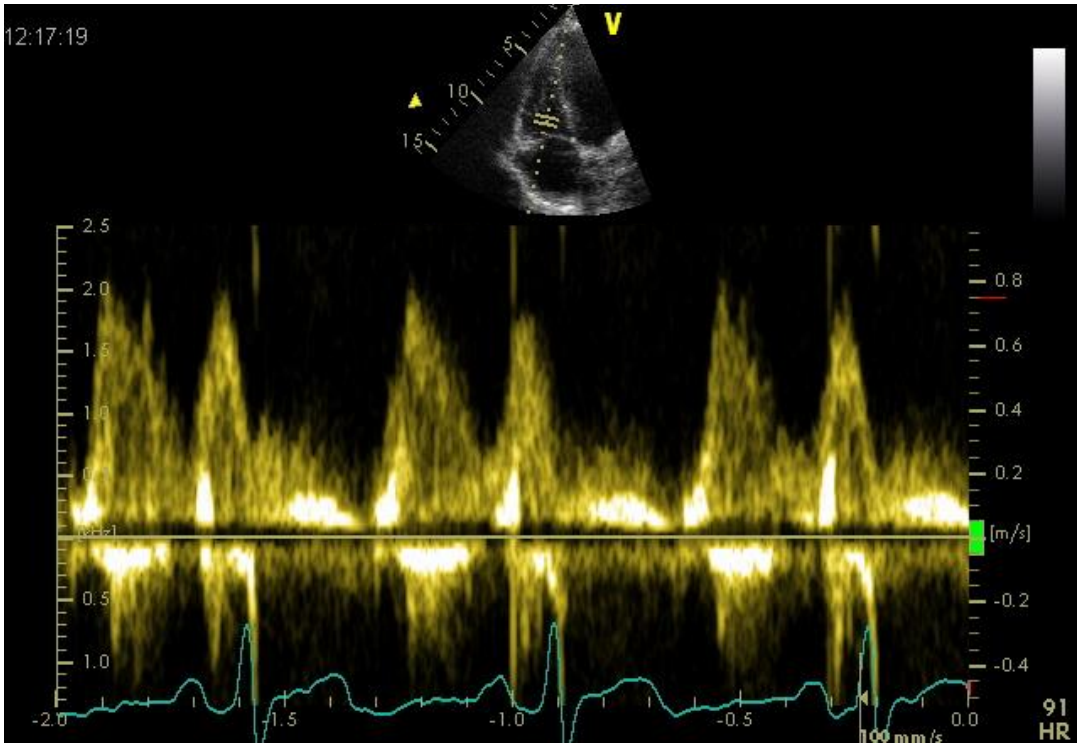


Figure 1.13 Pulsed wave Doppler of the TV, measuring forward flow across the valve

2.8.2. Tissue Doppler Imaging

From the A4C view, PW TDI was performed at both the TV annulus and at the MV annulus. A 2mm PW sample was used to detect peak myocardial velocities in order to obtain detailed information on both systolic and diastolic function of the RV and LV respectively. The sample window was placed in the basal lateral wall of the LV and the basal lateral wall of the TV. A high frame rate >150fps was used. The subject was asked to maintain gentle breathing and then pause

whilst the signal was acquired. Sweep speed, baseline and gain was adjusted to ensure clear timing and peak velocities. Care was taken not to overgain the S' (shown in Figure 2.10) velocity during post processing (Rudski et al., 2010)

2.8.3. Ratiometric and Allometric Scaling

In order to establish the effects that several common measures of height and body mass combinations have on normal RV variables, both ratiometric and allometric scaling techniques were employed. The standard ratiometric approach typically involves dividing the cardiac dimension in question by some anthropometric measurement. Four common body size variables were examined. These were height, body mass, BSA and BMI.

The aim of this simple linear scaling process (y =a/x), (where a equals calculated measure i.e. RVOT-1, and x equals the scaling variable such as BSA), is to remove the influence of the applied body dimension. For example, the resulting value from RVD-3/BSA, may help reduce some disparity between certain groups such as athletes that may vary with the associated body size variable. In addition to this form of simple scaling, allometric scaling has been suggested as an alternative method that accounts for the non-linear pattern of both cardiac dimensions and body size parameters (Batterham et al., 1999).

Each of the ratiometric variables was assessed for linearity to establish if they conformed to the Tanners Special Circumstance (TSC) and thus confirming standard ratiometric scaling as sufficient to remove any association with the indexed variable. The process was adequately described by (Batterham et al., 1999).

'that the coefficient of variation of the body size variable (CoVx) divided by the coefficient of variation (CoVy) of the chamber size is equal to the Pearsons correlation of the two variables (r,X,Y) CVx/CVy=r,x.y' (Batterham et al., 1999).

Where the associated correlation between these two measures was less than 0.3 it was assumed that little or no difference existed and therefore the results conformed to Tanners 'special circumstance' and as such would gain little from undergoing allometric scaling as a process (Batterham et al., 1999). Where these

two values were not similar, however, then there was no linear relationship in the distribution, with this typically presenting with either a positive or negative y intercept (George et al., 1998). In this case, the dimension in question would then be scaled using allometric techniques.

Allometric methods use the $y=a.x^b$ equation to investigate the relationship between RV 2D dimensions, 3D volumes and body size measures BSA, BMI, body mass and height using a non linear protocol within the statistics program SPSS (SPSS v20.0, SPSS Inc., Chicago, IL, USA) the Lavenberg-Marquardt algorithm. The calculated exponents (beta *b*) were defined by *'working in the arithmetic space defined by the original, raw X and Y variables'*(Oxborough et al., 2009). Multiple small consecutive adjustments are made to the parameter estimate until a global solution is found (Oxborough et al., 2009).

For each variable that did not conform to TSC, a size exponent (b) was calculated. This allowed the standard ratiometric methods to be powered appropriately for the non-linear nature of the dataset. To assess the impact of ethnicity on RV dimensions, independent of body size, a second assessment in addition to anthropometric data was undertaken using the model

Equation 3 Non-linear equation calculating the associated impact of ethnicity.

$$y = a{:}x^b.\exp(c.\text{ethnicity}),$$

Where a and x are as previously described and c is the calculated exponent derived for ethnic influence on RV dimensions in a body size independent group. The calculated results of the b values obtained from both equations were compared to assess the impact of the c values derived from the extended model.

2.9.RV Speckle Tracking

An RV focused view with a frame rate between 50 – 90 frames per second (fps) was acquired. A region of interest (ROI) was allocated to the whole of the RV septal and lateral wall. The ROI, shown within the RV was determined by placing markers along the septal endocardial border into the RV apex and the lateral wall, joining the TV annulus (see Figure 2.14).

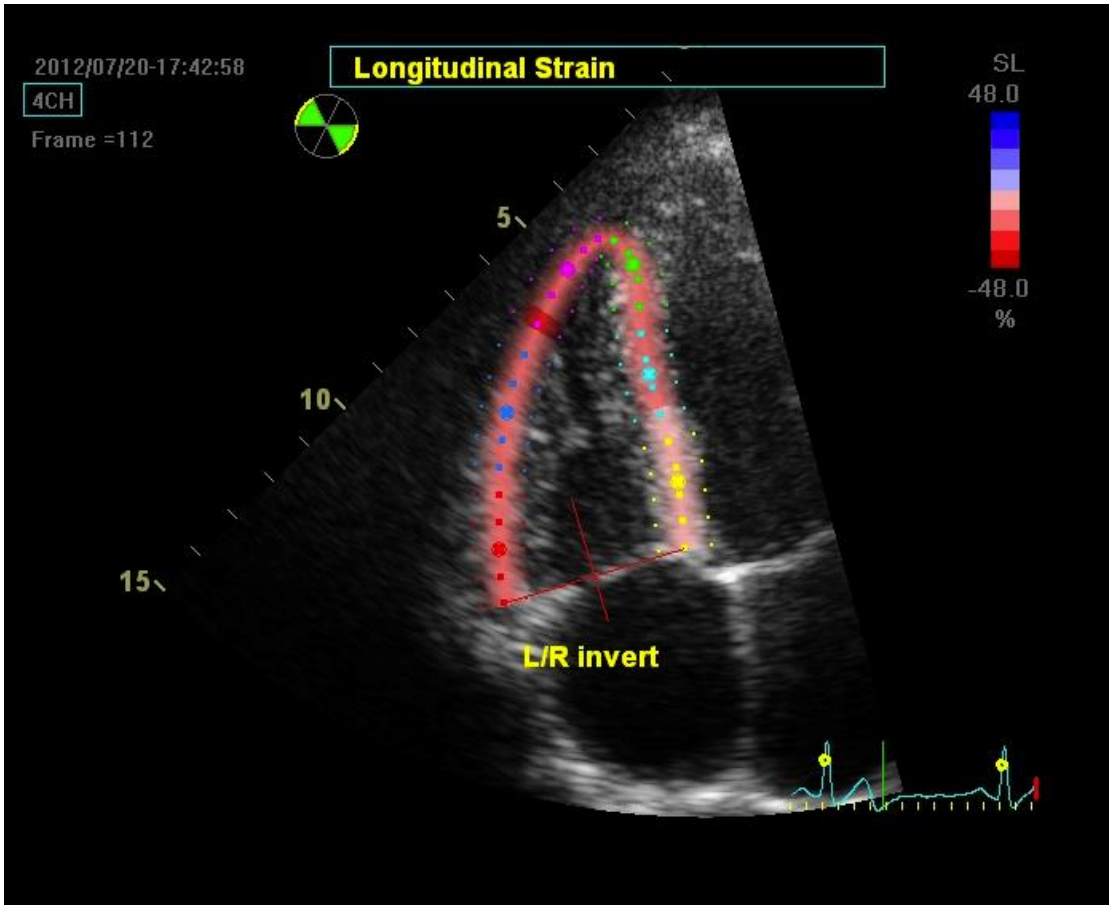


Figure 2.14 The RV Region of interest from the A4C view.

The width was adjusted to ensure sufficient cover of both the epicardial and endocardial borders. RV myocardial deformation was then calculated using frame-by-frame tracking of the 'natural acoustic markers' within a fixed area or 'kernel' (Korinek et al., 2005). Suitable tracking was confirmed via both the tracking algorithm and visual assessment of the ROI throughout the cardiac cycle. This ensured that normal myocardial thickening occurred in time with the cardiac cycle and no redundant or extra-cardiac tissue was tracked.

Studies were excluded from the speckle tracking arm of the study if the image quality restricted satisfactory tracking of the lateral RV wall in the apical four chamber view.

All images were conducted with the volunteer holding their breath so as to minimise translation movement. A detailed description of the tracking process has been previously described, in brief, however an algorithm calculates the sum-absolute-difference based on the best matched displacements between regions in two frames (Bohs and Trahey, 1991).

The images were reviewed for accurate thickening and longitudinal tracking throughout the cardiac cycle before approval was given. Individual segmental analysis was conducted to ensure correct assessment of both systolic and diastolic components.

Figure 2.15 RV speckle tracking echocardiography displaying tracking of both the septal and RV freewall as a six segment 'global' longitudinal strain curve. The horizontal dotted white line represents the global measurement derived from the six coloured traces. The vertical dashed lines shows the approximate measurements of peak systolic strain (PSS) (%).

Figure 2.16 RV speckle tracking echocardiography displaying tracking of both the septal and RV freewall as a six segment 'global' longitudinal strain curve. This trace displays both the peak systolic strain rate (PSR) as the negative systolic deflection with both early (SRe) and late (SRa) strain rate diastolic components displayed as a positive deflection. The dotted white line represents global strain rate.

Systolic measurement of peak systolic strain (PSS) (figure 2.15) was defined as the most negative deflection to occur prior to pulmonary valve closure (PVC) for each of the six individual segments with peak systolic strain rate (PSSR) (figure 2.16), calculated as the most positive deflection prior to PVC. Diastolic components, early strain rate (SRe) and late strain rate (SRa) were defined by negative deflections occurring in the early and late diastolic SR period respectively.

Additional strain and strain rate values were measured using the three basal, mid and apical lateral wall segments. This was reported as RV freewall and allowed for comparison of global versus lateral only measurements in an effort to replicate previous work conducted on the RV (Oxborough et al., 2012b, Teske et al., 2009a, Tong et al., 2008).

2.10. 3D Volume analysis

Real time 3D images used for volumetric assessment of the RV were acquired using a 3D matrix array probe. This operates at a lower frame rate than the standard 2D probe due to the combination of multiple acquisitions at the same time. These images are then built up over a series of 4-5 cardiac cycles with the patient gently holding their breath and then stitched together to produce a 3D block of data.

2.10.1. Acquisition

The standard A4C sometimes presents a challenging view from which only a limited lateral resolution of the RV freewall can be obtained, limiting acquisition of both 2D and 3D data. In order to minimise this the position of the patient was adjusted from the standard left lateral decubitus position to a more supine approach, moving the heart to a more medial position. By moving the RV to a more medial position within the sector, this lateral drop out can be improved (van der Zwaan et al., 2011a).

This manoeuvre creates a specific RV focused view for both 2D and 3D measurements ensuring maximal RV dimensions are recorded (Rudski et al., 2010). Gated to the ECG and with the patient holding their breath, the dataset is acquired over four beats and stitched together. This helps to minimise the lower frame rates presented by 3D evaluation although limits assessment in those with frequent ectopic beats or Atrial Fibrillation.

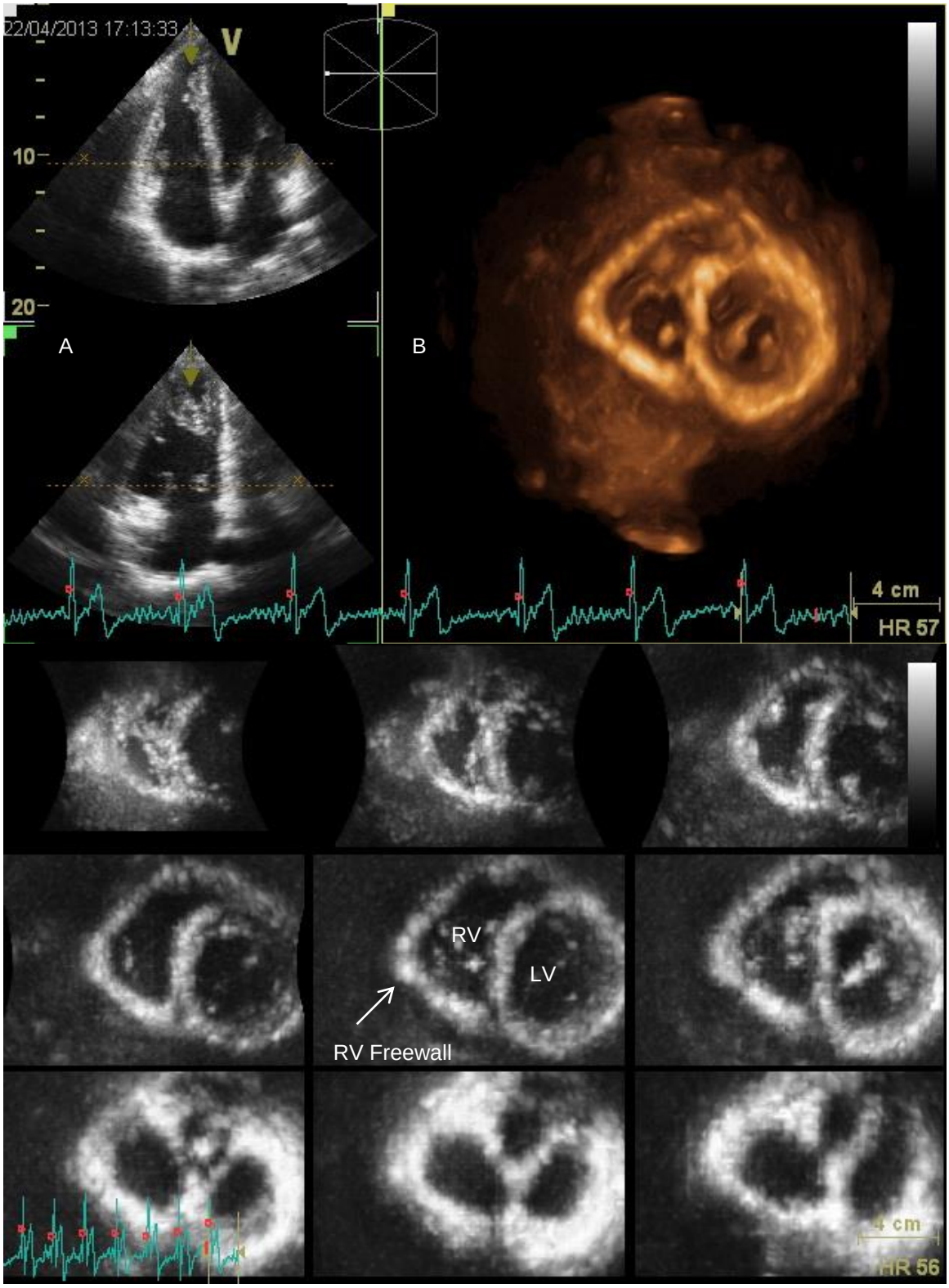


Figure 2.17 A 3D acquired acquisition of the RV and LV using the 3D matrix array probe. The top picture displays the visual assessment profile taken at source showing both a apical view (A) and a short axis view (B). This produces the 9-slice assessment view (bottom) which presents the stacked images of the LV and the RV built up over several gated ECG cycles.

Post-acquisition assessment of the image quality was made using a 9-slice view that breaks the 3D data into nine short axis windows (Figure 2.17). Here assessment of the RV apex, lateral wall and potential stitch artefact can be made. The stitch artefact represents poor delineation between adjoined datasets as the four beat cycle builds the 3D model. Sudden alterations in rhythm or movement can cause a jump in the acquisition process leaving a scar, described as a stitch, across the whole dataset. Despite the assessment in multiple views shown in figure 2.18, key components of the RV remain "unseen" during acquisition and can only be assessed once the data has been moved to the third party software.

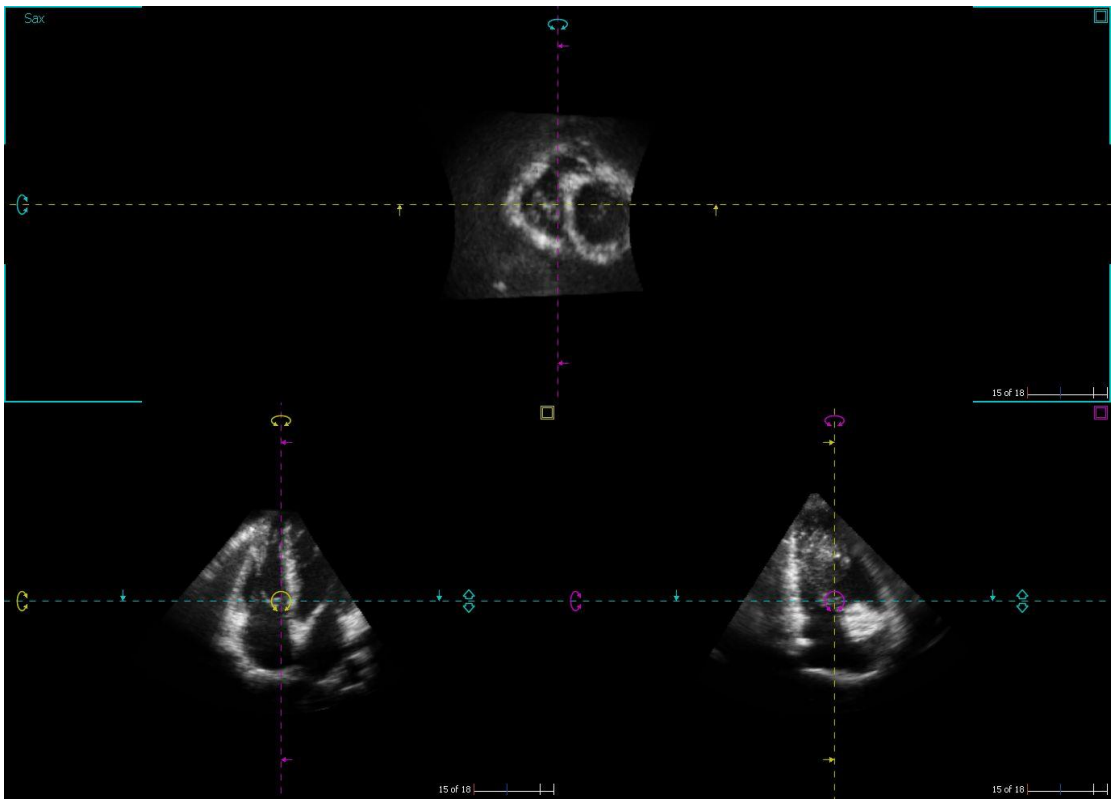


Figure 2.18 A modified view of the 3D acquisition presented in the TomTec software. This is produced from the images displayed in Figure 2.17 where the 'block' of 3D data is displayed within the RV specific software package.

2.10.2. TomTec

A brief description of the techniques used to analyse RV volumes follows:- Dedicated software for RV analysis was used for assessment of RV volumes and ejection fraction (4D RV-Function©, TomTec Imaging Systems, Unterschleissheim, Germany). Analysis was conducted in three planes – apical 4

chamber, sagital and coronal. Once the location of the TV, MV and apex had been identified, the software programme defines both end diastole and end systole. Based on the location of the TV, MV and apex, both the A4C and coronal view are automatically defined, as shown in figure 2.19.

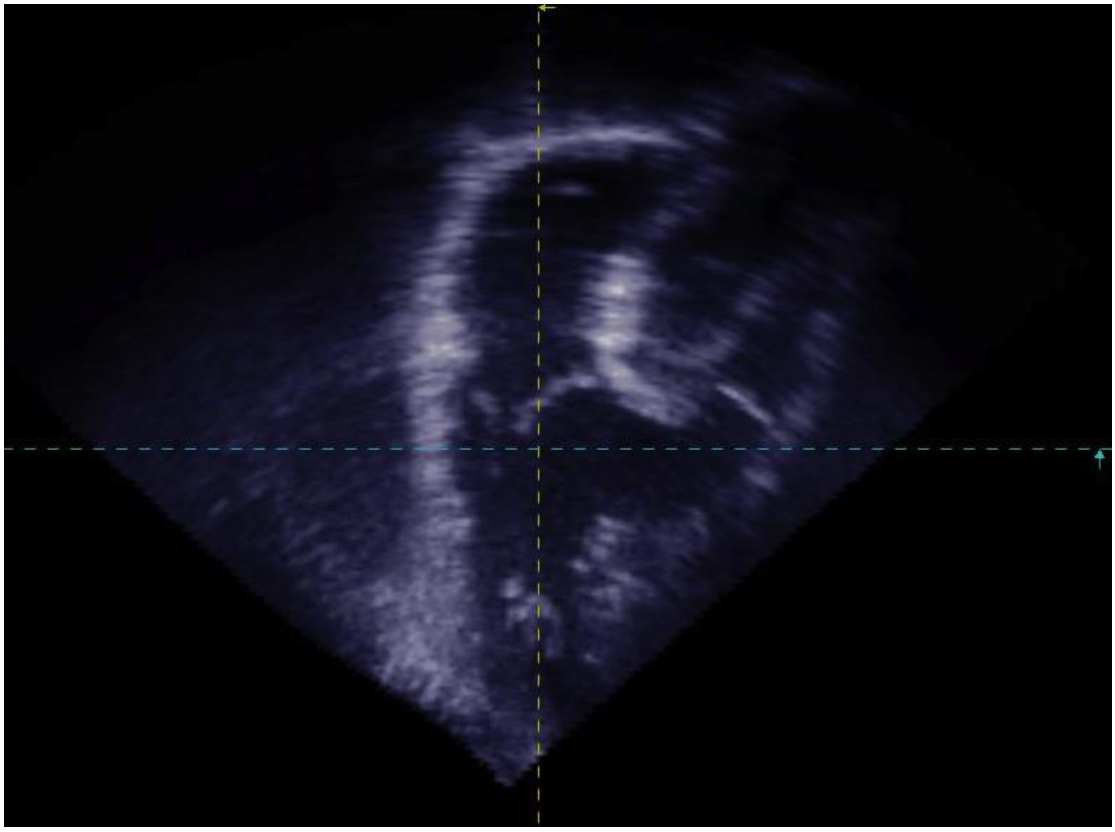


Figure 2.19 Coronal view presented post acquisition, within the 3D software programme using the data displayed within Figure 2.17.

Manual tracking of the endocardial border is then required at both time phases in all views. Once verified, tracking of the RV was used to calculate end systolic volume (ESV) end diastolic volume (EDV) ejection fraction (EF) and stroke volume (SV). A 3D volume (Figure 2.20) model is also produced that unlike other assessment methods, does not rely as heavily, on geometrical assumptions (Jiang et al., 1997). A volume time curve displays the ejection of blood throughout the cardiac cycle.

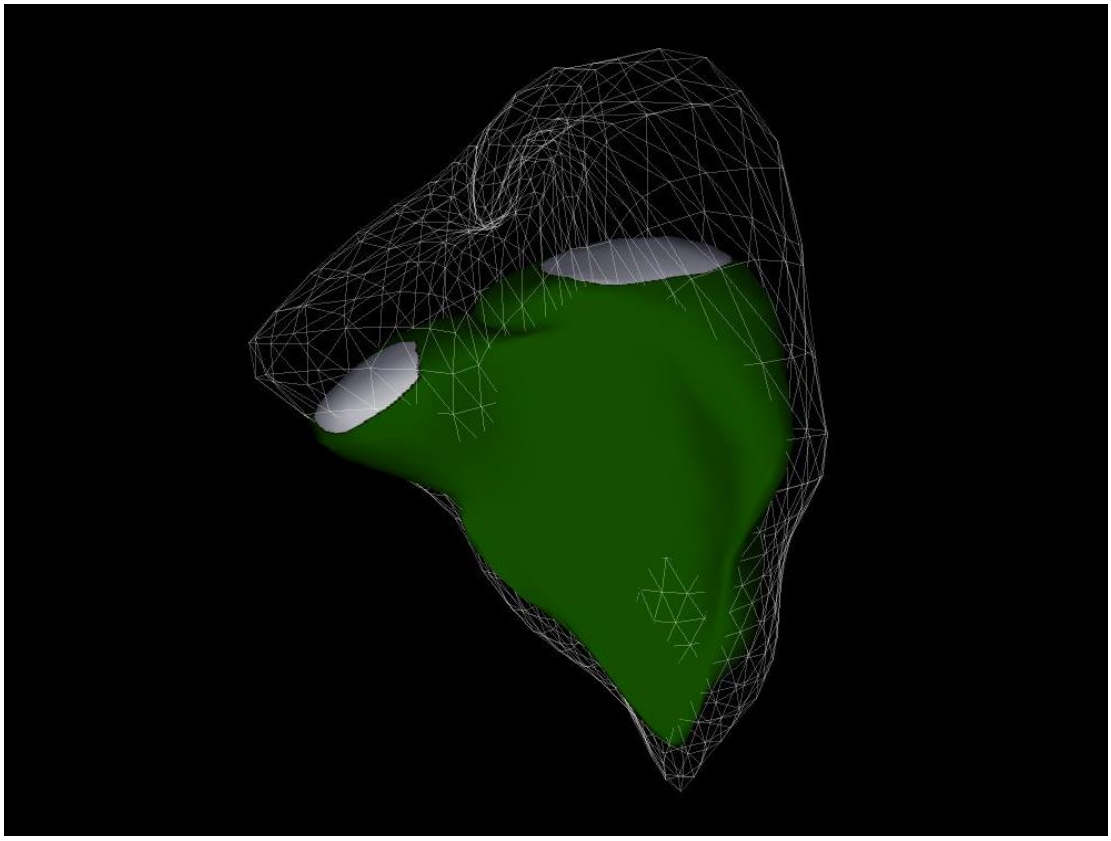


Figure 2.20 3D volumetric model of the RV.

2.11. Statistical analysis

Measurements across each of the imaging modalities, taken from the original images were referred to as raw data. This data was input into the software package IBM SPSS statistics version 20. Linear dimensions were measured three times with the average measurement taken. All measurements were assessed for normality using a visual assessment of histogram data, P-P plots and residual analysis (Field, 2013).

Data that conformed to a normal distribution were presented as mean + SD or were log transformed for analysis if appropriate. Reference intervals were calculated as Mean + 1.96SD, giving a 95% upper and lower reference limit (Bland and Altman, 1999).

Assessment of raw data and subsequent scaled datasets was conducted using independent t-test for gender, analysis of variance (ANOVA) to assess for

intergroup differences. Tukeys honestly significant difference test was used to establish where intergroup differences might lie.

Multiple regression modelling was used to better understand the relationship between the dependant variables (RV dimensions, strain, strain rate and 3D volumes) and several common independent variables (age, gender, ethnicity height and body mass). By controlling for the independent variables, it was possible to ascertain what significant relationships existed. This was tested on several occasions using varying methods for calculating size independent data-sets.

RV size has previously been demonstrated to be independent from LV size (Kawut et al., 2011). This was tested in a second adjusted regression model for simple RV linear measurements by including LV diastolic internal dimension (diastolic) (LVIDd) within the covariants.

Reference variables were chosen as a basis for comparison within the regression modelling. These were female gender, European ethnicity, and $\leq$29 years. Results from the remaining groups were compared to this reference standard to provide details on the extent of the relative change in size or demographic by comparison. This technique was featured within a number of studies produced by the MESA group (Kawut et al., 2011, Fernandes et al., 2011).

3. Results

Over the duration of the study, 302 volunteers were prospectively recruited from three centres. From that group 255 volunteers underwent a full echocardiogram study. Optimal images were acquired where possible to obtain 2D, 3D and speckle tracking data sets.

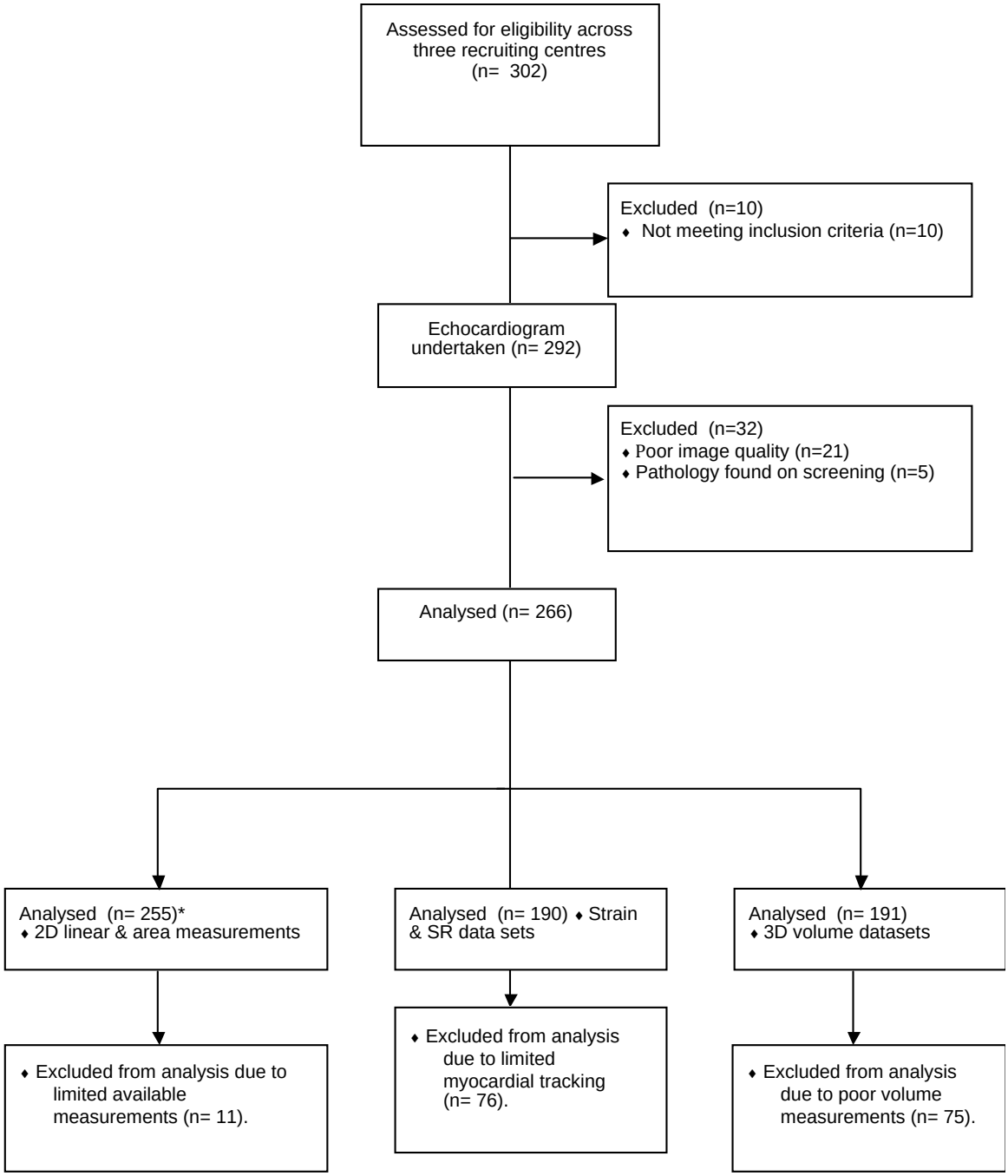
53% of the study population were male. Five ethnic groups were included within the final study group – Afro-Caribbean (n = 49) European (n = 46) Chinese (n = 66) Indian (n = 45) and Malay (n = 49). Resting systolic blood pressure was 115±12mmHg over 71±9mmHg diastolic in the European group and 124±13mmHg over 77±7mmHg in the Afro-Caribbean group. All remaining volunteers in the Indian, Chinese and Malay groups were normotensive with a resting blood pressure of (<140/90mmHg). Basic anthropometrics of the study group can be seen in Table 3.1. The results section contains general results about the study demographic, with specifics pertaining to each of the three testing areas described later.

The mean age was 41 ±11 yrs. Ages across the groups ranged from 19 years to 73 years. The distribution between the different age groups for the entire study cohort (n = 266) was as follows with n = 55 in the ≤29 age group, n 51 = ≤39 age group, n = 94 ≤49 age group, n = 44 ≤59 age group and n = 22 in the 60+ group.

3.1. Recruitment

The main source of recruitment came from word of mouth with an approximate 50/50 mix of hospital staff and friends and family. The Malay, Chinese and Indian sample was recruited via the Gleneagles Medical Centre, Penang, Malaysia. Volunteers were recruited from the local area with hospital staff, friends and family and local clubs and social groups participating. Afro-Caribbean volunteers were recruited via the Royal Free Hospital in London. The study was advertised within the hospital with employees, friends and family forming the study group. European volunteers were recruited from staff friends and family via the Royal United Hospital using a similar process.

Five sonographers were involved at various stages, in the acquisition of study images (JE, DA, JS, AS & JAW). In total >70% of echocardiograms were performed by the author (JAW). A team of three sonographers (JAW, DA & JE) undertook scans within Gleneagles hospital, Malaysia. The UK scans at the Royal United Hospital, Bath (JAW & JS) and the Royal Free Hospital in London, were then performed by the author, with two additional sonographers (JAW, JS & AS).



* Measurement acquisition is reported in Table 3.3

Figure 3.0 CONSORT diagram displaying the distribution of recruited volunteers and successful scans across each of the three RV assessment techniques.

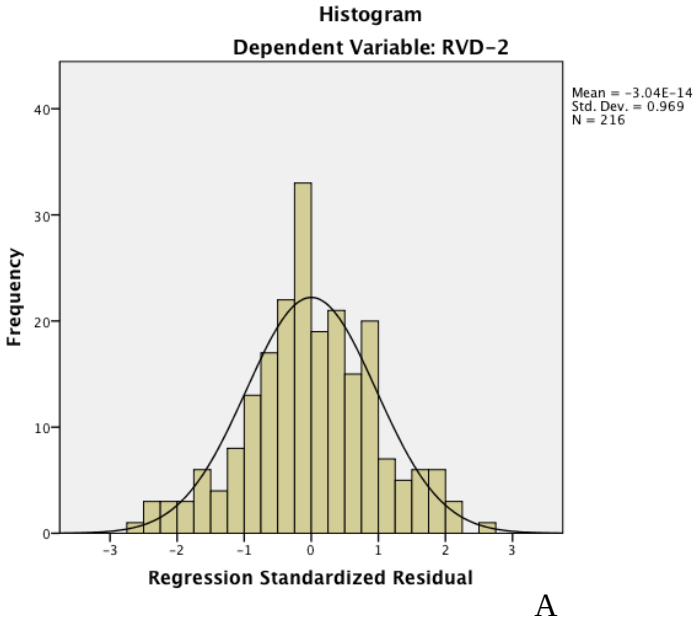
The author was present at all centres, performing the vast majority of scans to minimise interobserver acquisition however the use of repeat acquisitions was undertaken to ensure that this variable could be accounted for as a study variable within the repeatability section. The breakdown of volunteers into the study is displayed in Figure 3.0 with those excluded at each stage noted.

3.2. Study Distribution

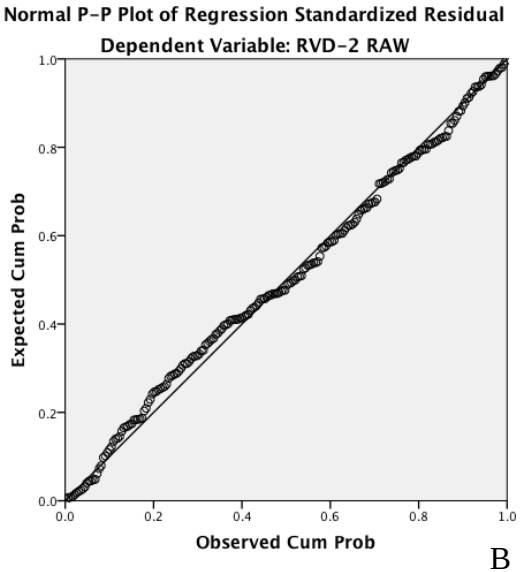
Due to the large sample size, assessment of normality via pre-programmed tests such as the Kolmogorov-Smirnov test was considered insufficient. Instead, the data was visually investigated for features of normality visually, using both frequency and residual analysis (Field, 2013).

Assessment of the raw data was conducted to ensure that it conformed to a normal distribution. Histograms were calculated to assess for outliers. Cumulative probability of each dimension was plotted against the probability of conforming to a normal distribution using P-P plots. The graphed results were assessed for any departures from normality. The standardized residuals were plotted using a histogram and scatter graph against the predicted (fitted) values for each of the ten measurements. For all eight linear measurements and two area measurements the residuals displayed an appropriate random distribution and therefore conformed to the assumptions of linearity and homoscedasticity.

Assessment of the strain and strain rate characteristics were conducted in a similar fashion. The raw data was investigated for any significant departures from normality using the methods described above. Strain, Strain rate and both early and late diastolic SR were all found to conform to a normal distribution. Each of the four 3D measurements also conformed to a normal distribution. Figure 3.1 shows an example of a normal distribution histogram, P-P plot, and a scatter plot of residuals for RVD-2.



A



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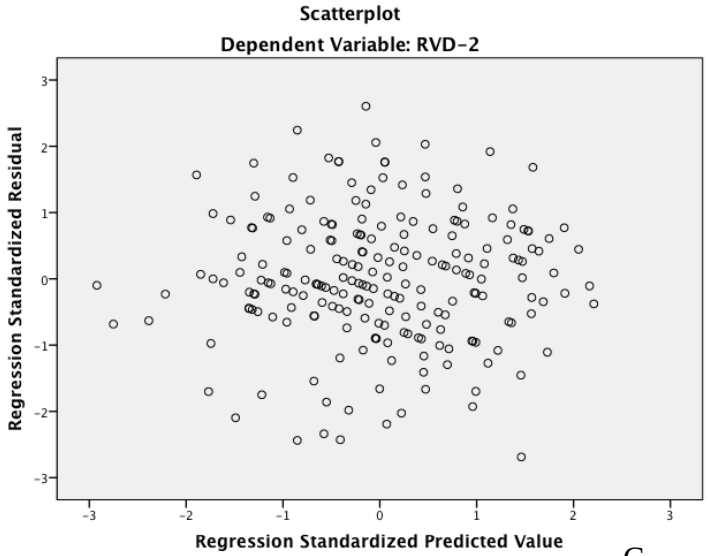

C

Figure 3.1 Sample graphs from linear measurements showing (A) normal distribution of residuals (B) P-P plots and (C) random scatter of the residuals.

Table 3.1 Basic anthropometric data organised by gender and ethnic group

	Height (cm)	Body Mass (kg)	BSA (m2)	BMI (kg/m2)
All	166.5 ± 9.6	71.3 ± 15.3	1.88 ± 0.2	25.6 ± 4.5
Female	160.5 ± 7.9	66.4 ± 15.7	1.68 ± 0.2	25.6 ± 5.1
Male	171.8 ± 7.6	75.6 ± 13.6	1.9 ± 0.2	25.6 ± 4.0
Afro-Caribbean	170.4 ± 7.7	79.9 ± 15.0	1.93 ± 0.2	27.5 ± 4.9
Chinese	163.5 ± 10.0	65.1 ± 15.3	1.71 ± 0.2	24.2 ± 4.3
European	171.5 ± 10.2	74.9 ± 14.0	1.87 ± 0.2	25.3 ± 3.2
Indian	164.5 ± 8.2	68.2 ± 13.8	1.75 ± 0.2	25.2 ± 4.9
Malay	163.0 ± 8.0	68.6 ± 13.0	1.75 ± 0.2	25.8 ± 4.1

Table 3.1 shows the basic anthropometric demographics organised by both gender and ethnicity. European (171.52±10.15 cm) and Afro-Caribbean (170.38±7.72cm) groups, despite showing no significant difference, were taller ($p<0.01$) compared to Malay (163.02±8.04cm), Indian (164.54±8.18cm) and Chinese (163.46±10.03cm) groups. The Afro-Caribbean group was heaviest (79.86±15.03 kg, $p<0.01$).

This was reflected in the calculated BSA with the Afro-Caribbean group showing an increased BSA ($p<0.0001$, 1.93±0.20m^2) compared to Malay (1.75±0.19m^2), Indian (1.75±1.9m^2) and Chinese (1.71±0.22m^2). European (1.87±0.21m^2) BSA also exceeded that of Malay, Indian and Chinese ($p<0.05$). ANOVA results revealed a significant difference ($p<0.01$) between ethnicities for height (cm) body mass (kg), BSA and BMI.

3.3.LV measurements

Table 3.2 Basic left and right sided echocardiographic measurements with both male and female results for comparison

Measure	All	*n*	Male	*n*	Female	*p*
IVSd (cm)	0.8 ± 0.2	126	0.9 ± 0.1	111	0.7 ± 0.1	<0.0001
LVIDd (cm)	4.7 ± 0.5	126	4.8 ± 0.5	111	4.5 ± 0.4	<0.0001
LVIDd/BSA (cm/m^2)	2.6 ± 0.3	126	2.6 ± 0.3	111	2.7 ± 0.3	0.02
LVPWd (cm)	0.8 ± 0.1	126	0.8 ± 0.1	111	0.7 ± 0.1	<0.0001
LVIDs (cm)	2.9 ± 0.5	124	3.0 ± 0.5	110	2.7 ± 0.4	<0.0001
LVIDs/BSA (cm/m^2)	1.6 ± 0.3	124	1.6 ± 0.3	110	1.6 ± 0.3	ns
EF Biplane (%)	64 ± 5.3	111	63 ± 5.4	94	64 ± 5.3	ns
LV AP (cm)	4.4 ± 0.5	120	4.5 ± 0.5	95	4.2 ± 0.5	<0.0001
LV SL (cm)	4.3 ± 0.5	120	4.4 ± 0.5	95	4.1 ± 0.4	<0.0001
Eccentricity Index	1.0 ± 0.1	120	1.0 ± 0.1	95	1.0 ± 0.1	ns
LA Volume A-L (cm^3)	40 ± 11.6	97	41 ± 11.0	82	39 ± 12.3	ns
RA Voume A-L (cm^3)	33 ± 12.3	93	37 ± 12.3	78	29 ± 10.8	<0.0001
HR (bpm)	67 ± 11.7	126	66 ± 11.6	111	68 ± 11.8	ns
MV E Vel (m/s)	0.7 ± 0.1	126	0.7 ± 0.1	108	0.8 ± 0.1	<0.0001
MV DecT (ms)	173 ± 37.0	126	176 ± 38.4	108	169 ± 35.1	ns
MV Dec Slope (m/s)	4.5 ± 1.4	126	4.2 ± 1.3	108	4.9 ± 1.4	<0.0001
MV A Vel (m/s)	0.5 ± 0.1	126	0.5 ± 0.1	108	0.6 ± 0.2	0.001
MV E/A Ratio	1.4 ± 0.4	126	1.4 ± 0.4	108	1.4 ± 0.4	ns
LV S Wave (m/s)	0.1 ± 0.1	118	0.1 ± 0.1	102	0.1 ± 0.02	ns
E' lateral MV (m/s)	0.1 ± 0.03	121	0.1 ± 0.03	101	0.1 ± 0.03	ns
E/e' Mitral	6.0 ± 1.7	119	5.8 ± 1.7	99	6.2 ± 1.6	0.04
MV A Wave (m/s)	0.1 ± 0.02	121	0.1 ± 0.02	102	0.1 ± 0.02	ns
E' septal (m/s)	0.1 ± 0.02	118	0.1 ± 0.02	99	0.1 ± 0.02	ns

LV measurements (shown in Table 3.2) demonstrated a normal range for the total group and each gender respectively. Male results for LV wall thickness and internal dimensions exceeded that of females ($p<0.0001$). There was no significant difference in ejection fraction. Simple indexing of LV measurements to BSA showed a

significant difference ($p<0.05$) in LVIDd/BSA but no significant difference in LVIDs/BSA between genders. LV internal dimensions demonstrated a normal distribution. TV inflow, deceleration time and estimated peak systolic pulmonary artery pressure (PSPAP) demonstrated no significant difference between males and females (ns).

3.4. RV Linear dimensions

3.4.1. 2D Measurement Acquisition

Measurement acquisition refers to the number of adequate images obtained across each of the linear and area dimensions. This varied across each ethnic group. Table 3.3 shows the distribution between groups for each of the ten basic RV measurements. These ranged from 98% to 76% in the PSAX images (RVOT-1 & 2), 65% to 87% in the A4C view (RVD-AN, RVD-1 to 3) and 79% to 98% in the PLAX view (RVOT-3). Apical end diastolic and end systolic area measurements ranged from 65% to 81%.

The Malay population consistently displayed the lowest attainable percentage of measurement acquisition in the apical window while the Afro-Caribbean displayed the highest. This is discussed in brief in section 4.1. Afro-Caribbean and European volunteers displayed the lowest attainable measurements in RVOT-1 and RVOT-2 respectively. Both RVOT-3 and RV-WT were the most attainable measurements for the group as a whole with both PLAX images attained in 90% of the study population.

Table 3.3 Number and percentage of measurement acquisitions across each linear dimension and ethnic group.

Ethnicity	Total (n)	RVOT-1		RVOT-2		RVOT-3		RV-WT		RVD-1		RVD-2		RVD-3		RVD-AN		RV-EDA		RV-ESA	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Afro-Caribbean	49	46	(94)	46	(94)	46	(94)	46	(94)	46	(94)	49	(100)	46	(94)	46	(94)	47	(96)	47	(96)
European	46	38	(83)	35	(76)	42	(91)	42	(91)	37	(80)	40	(87)	38	(83)	38	(83)	36	(78)	36	(78)
Chinese	66	59	(89)	59	(89)	61	(92)	61	(92)	52	(79)	55	(83)	54	(82)	55	(83)	53	(80)	53	(80)
Indian	45	43	(94)	41	(89)	45	(98)	45	(98)	32	(70)	36	(78)	34	(74)	37	(80)	30	(65)	30	(65)
Malays	49	45	(94)	40	(83)	43	(90)	43	(90)	31	(65)	36	(75)	33	(69)	35	(73)	31	(65)	31	(65)
Total	255	231	(88)	221	(84)	237	(90)	237	(90)	198	(75)	216	(82)	205	(78)	211	(80)	196	(74)	196	(74)

Table 3.4 Simple RV linear measurements taken from the entire study cohort. Results are presented in mm+SD with upper and lower CI and 95% Reference range.

				CI		Reference range	
Measure	n	mean	sd	Lower	Upper	Lower	Upper
RVOT-1 (mm)	231	28 ±	4.39	27.9 -	29.0	20 -	37
RVOT-2 (mm)	219	21 ±	4.57	20.5 -	21.7	12 -	30
RVOT-3 (mm)	237	25 ±	3.72	24.9 -	25.8	18 -	33
RV-WT (mm)	237	4 ±	0.75	4.0 -	4.2	3 -	6
RVD-1 (mm)	194	32 ±	5.21	30.9 -	32.4	21 -	42
RVD-2 (mm)	213	30 ±	4.49	29.1 -	30.3	21 -	39
RVD-3 (mm)	205	74 ±	7.28	72.8 -	74.8	59 -	88
RVD-AN (mm)	211	25 ±	5.12	24.1 -	25.5	15 -	35
RVEDA (cm^2)	193	17 ±	4.54	16.0 -	17.3	8 -	26
RVESA (cm^2)	193	9 ±	2.78	8.3 -	9.1	3 -	14
RVFAC	193	48 ±	7.04	46.7 -	48.7	33.9 -	61.5
TAPSE	224	2 ±	0.31	2.3 -	2.3	1.7 -	2.9
RV S Wave	225	13 ±	1.88	12.8 -	13.4	9.4 -	16.7

3.4.2. Simple 2D linear and area measurements

Each of the eight linear, two area, and functional measures of the RV described within the method section were calculated as an average of three measurements. Basic results including mean, standard deviation (SD), reference ranges and 95% Confidence Intervals (CI) on the simple linear data are presented in Table 3.4. Reference ranges were calculated using the measurement SD multiplied by 1.96. This was then added to, or taken away from the mean value of the measurement giving both the upper (URV) and lower reference vales (LRV).

RVOT-1 and RVOT-2 values for the study cohort ranged from 17mm – 40mm and 12mm to 30mm respectively. Apical measurements ranged from 20mm to 47mm in RVD-1, 56mm to 98mm in RVD-3, 18mm to 40mm in RVD-2 and 14mm to 41mm in RVD-AN. RV ESA and EDA ranged from 9.6cm^2 to 32cm^2 and 4.1cm^2 to 17,9cm^2 respectively. PLAX images ranged from 18mm to 39mm in RVOT-3 and 3mm to 6mm in RVWT.

Compared to the 2005 guidelines (Lang et al., 2005), the percentage of measurements outside the upper limit for normal reference values, ranged from 17% for RVD-3 to 69% for RVD-1. In comparison, current ASE guidelines (Rudski et al., 2010) saw a reduction from 69% to 3% for RVD-1. The number of studies with measurements in excess of these recent guidelines ranged from just 2% for RVOT-3 to 7% for RVEDA.

Table 3.5 Simple linear dimensions presented for each ethnic group. Results show measurements in mm+SD. ANOVA analysis indicates that at this stage significant differences between ethnic groups exist.

Measurement	Chinese	Malay	Indian	European	Afro-Caribbean	*P*
RVOT-1 (mm)	27 ± 3.75	27 ± 3.26	27 ± 3.66	32 ± 4.67	30 ± 4.16	<0.0001
RVOT-2 (mm)	21 ± 3.38	20 ± 3.08	20 ± 2.65	21 ± 3.01	23 ± 3.08	<0.0001
RVOT-3 (mm)	24 ± 3.19	24 ± 2.96	24 ± 2.99	28 ± 3.68	27 ± 3.26	<0.0001
RV-WT (mm)	4 ± 0.68	4 ± 0.73	4 ± 0.65	4 ± 0.78	5 ± 0.72	<0.0001
RVD-1 (mm)	30 ± 3.58	30 ± 3.23	30 ± 4.18	31 ± 4.22	37 ± 6.04	<0.0001
RVD-2 (mm)	29 ± 4.93	29 ± 3.77	29 ± 5.18	31 ± 4.60	31 ± 3.20	<0.006*
RVD-3 (mm)	72 ± 5.06	70 ± 4.81	71 ± 5.78	79 ± 7.08	76 ± 8.49	<0.0001
RVD-AN (mm)	22 ± 3.83	23 ± 3.95	22 ± 3.50	26 ± 3.66	30 ± 4.68	<0.0001
RVEDA (cm^2)	15 ± 2.90	14 ± 2.56	14 ± 2.63	18 ± 4.31	21 ± 4.49	<0.0001
RVESA (cm^2)	8 ± 2.03	7 ± 1.89	7 ± 1.73	10 ± 2.46	11 ± 2.89	<0.0001

Table 3.5 shows the RV simple linear dimensions organised by ethnic group. Initial assessments using a one way ANOVA were made for each dimension based on subject ethnicity. This revealed a highly significant difference ($p<0.01$) between ethnic groups, for each of the ten measurements. Typically, either the Afro-Caribbean or European group presented with the largest RV dimensions. When assessed using independent t-tests, results indicated that male measurements exceeded that of females ($p<0.01$) in nine out of the ten measurements. RV wall thickness showed no significant difference. Mean±SD male and female results are shown in Figure3.2 and 3.3, organised by echocardiographic window with mean values in Table 3.6.

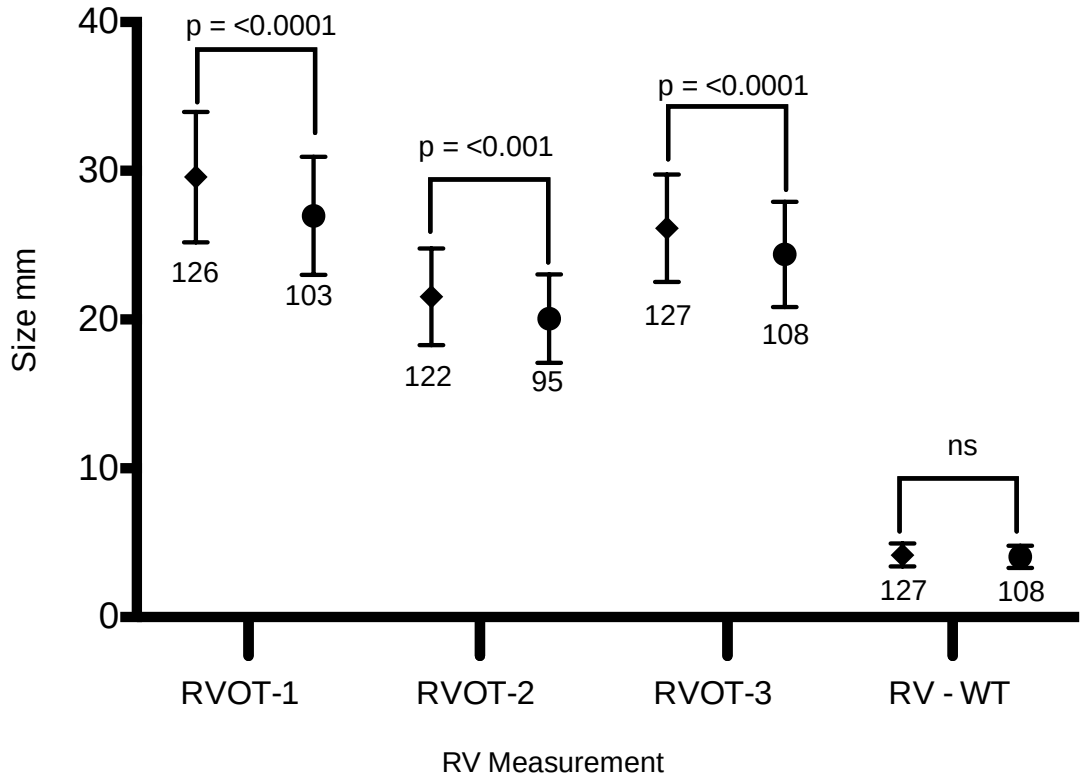

Figure 3.2 Mean±SD (n) parasternal RV measurements organised by gender. ◆= male ● = female. ns = non-significant.

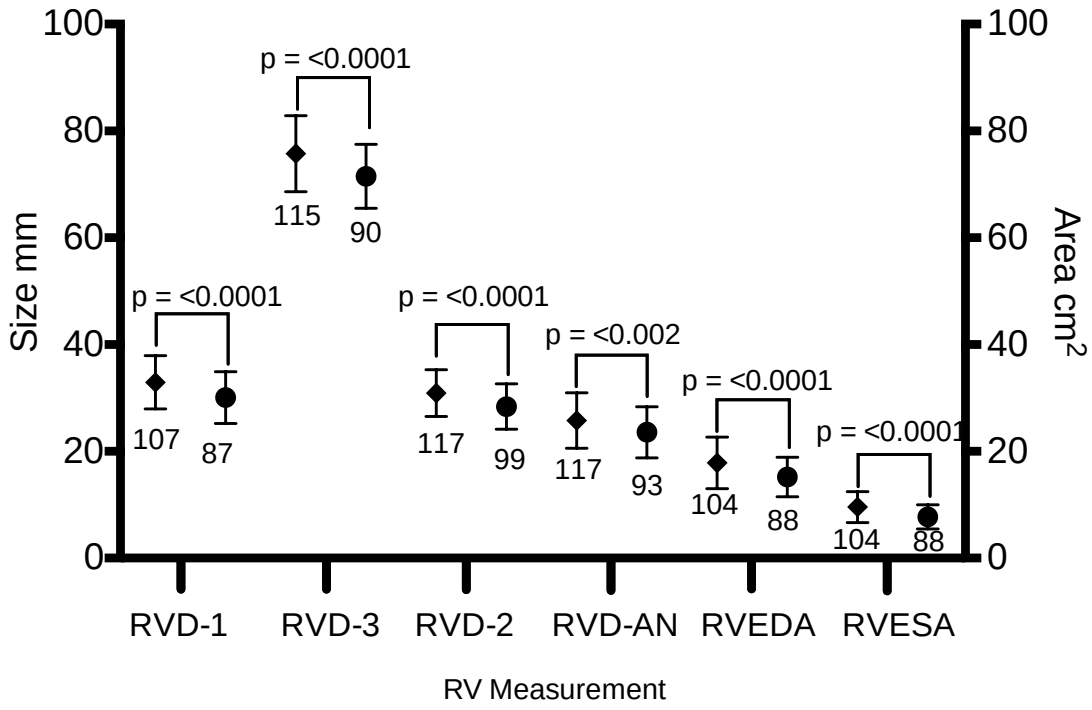

Figure 3.3 Mean±SD (n) apical RV measurements and area measurements organised by gender. ◆= male ● = female.

Table 3.6 Mean RV measurements (mm) organised by gender, displaying the highly significant differences between male and female dimensions.

	Male				Female				
	n	Mean		SD	*n*	Mean		SD	*p*
RVOT-1	126	30	±	4.4	103	27	±	4.0	<0.0001
RVOT-2	122	22	±	3.2	95	20	±	3.0	0.001
RVOT-3	127	26	±	3.6	108	24	±	3.6	0.0002
RV-WT	127	4	±	0.8	108	4	±	0.7	ns
RVD-1	107	33	±	5.0	87	30	±	4.8	<0.0001
RVD-2	117	31	±	4.4	99	28	±	4.2	<0.0001
RVD-3	115	76	±	7.1	90	71	±	6.0	<0.0001
RVD-AN	117	26	±	5.2	93	24	±	4.8	0.002
RV-EDA	104	18	±	4.8	88	15	±	3.7	<0.0001
RV-ESA	104	10	±	2.9	88	8	±	2.3	<0.0001

Tables 3.7 - 3.9 present the results of the regression analysis organised by image window. It is apparent that even after controlling for age, gender, height and body mass, ethnicity remains highly significant ($p \leq 0.006$) in every measurement except RVD-2. Compared to the European reference group, however, the association between ethnicities varies.

On average, the European group displayed statistically larger dimensions in every permutation than the Indian, Chinese and Malay populations, however the Afro-Caribbean group exceeded the European group in RVOT-2 (b =1.72mm p =0.01), RVD-1 (b=5.19mm p<0.001), RVD-AN (b=4.15mm p<0.001) and both area measurements (b =2.36cm^2 and b =1.62cm^2 p <0.01). The largest variation in size, (taken from the beta values in Table 3.7 with all other variables held constant), was 7.2 mm for RVD-3, between the European and Malay populations. Similar size variations were also found with the Chinese group.

Age was a significant covariant in a number of measurements but with limited impact across each of the age groups. Interestingly when compared to the ≤29 years age group results showed that on average RVOT measurements were larger with increasing age whilst apical dimensions tended to be smaller by comparison. Despite this there was limited statistical significance with RVOT-2 and 3 (≤49 years), RVD-3 (≤ 49 years and 60+ years), RV-WT (≤ 39 years and 60+ years) and RVEDA (≤ 49 years) showing significant results compared to the reference group.

Gender remained significant for all RVOT measurements, RVD-1 and RVD-3. Males showed (with all other variables held constant) a mean increase of up to 2.4mm compared to females. Associated adjusted R^2 values ranged from 15.7% for RV-WT to 53.6% for RVEDA.

Using a second regression model adjusted for LV size, which included LVIDd, no significant change was found across any of the eight linear measurements. The remaining significant associations (b) were on average, smaller with additional adjustment for LV size. RVEDA and RVESA, when adjusted for LV size, were no longer significant for body mass within the model.

Table 3.7 Multiple regression tables for simple linear RV measurements made in the parasternal view.

	RVOT-1 33.9%				RVOT-2 24.9%				RVOT-3 22.3%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference											
Male	2.43	1.15	3.71	0.001	1.18	0.11	2.24	0.03	1.19	0.21	2.17	0.02
Body Mass	0.08	0.04	0.12	<0.001	0.08	0.05	0.12	<0.001	0.11	0.07	0.14	<0.001
Height	-4.92	-12.86	3.02	ns	-4.15	-10.62	2.31	ns	-3.88	-10.06	2.29	ns
< 29 yrs[1]	Reference											
<39 yrs	0.48	-1.03	1.99	ns	0.52	-0.70	1.73	ns	0.60	-0.59	1.77	ns
<49 yrs	1.27	-0.11	2.65	ns	1.41	0.29	2.53	0.01	1.20	0.14	2.26	0.03
<59 yrs	0.84	-0.86	2.55	ns	1.00	-0.35	2.35	ns	1.11	-0.20	2.42	ns
60+ yrs	0.61	-1.39	2.62	ns	1.28	-0.34	2.90	ns	1.62	0.04	3.19	0.04
European	Reference											
Malay	-5.97	-7.70	-4.24	<0.001	-0.70	-2.17	0.77	ns	-4.52	-5.87	-3.18	<0.001
Chinese	-5.94	-7.65	-4.23	<0.001	-0.95	-2.38	0.47	ns	-4.65	-5.96	-3.34	<0.001
Indian	-4.63	-6.24	-3.03	<0.001	-0.02	-1.36	1.32	ns	-3.73	-4.96	-2.49	<0.001
AfroCar	-2.68	-4.29	-1.07	<0.001	1.72	0.39	3.06	0.01	-1.49	-2.73	-0.26	0.02
p	p<0.0001				p 0.006				p<0.0001			

[1] Age group sample size: RVOT-1 ≤ 29 yrs n 52; ≤ 39 yrs n 45; ≤ 49 yrs n 81; ≤ 59 yrs n 33; 60+ n 19. RVOT-2 ≤ 29 yrs n 51; ≤ 39 yrs n 43; ≤ 49 yrs n 73; ≤ 59 yrs n 33; 60+ n 18. RVOT-3 ≤ 29 yrs n 53; ≤ 39 yrs n 45; ≤ 49 yrs n 83; ≤ 59 yrs n 36; 60+ n 19.

Table 3.7 (cont) Multiple regression tables for simple linear RV measurements made in the parasternal view.

	RV-WT 15.7%			
	b	95% CI		*p*
Female				
Male	0.05	-0.20	0.29	ns
Mass	0.01	0.00	0.02	ns
Height	-0.15	-1.67	1.37	ns
≤ 29 yrs[2]				
≤39 yrs	0.39	0.10	0.68	0.01
≤49 yrs	0.22	-0.05	0.48	ns
≤59 yrs	0.20	-0.12	0.52	ns
60+ yrs	0.62	0.23	1.01	<0.001
European				
Malay	-0.54	-0.87	-0.21	<0.001
Chinese	-0.58	-0.90	-0.26	<0.001
Indian	-0.22	-0.52	0.08	ns
AfroCar	0.09	-0.22	0.39	ns
p	p<0.0001			

[2]Age group sample size: RVWT ≤ 29 yrs n 53; ≤ 39 yrs n 45; ≤ 49 yrs n 83; ≤ 59 yrs n 36; 60+ n 19.

Table 3.8 Multiple regression tables for simple linear RV measurements made in the apical view

	RVD-1 38.0%				RVD-2 17.0%				RVD-3 41.8			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female												
Male	1.92	0.30	3.54	0.02	1.03	-0.48	2.54	ns	2.38	0.37	4.39	0.02
Body Mass	0.08	0.03	0.14	<0.001	0.06	0.00	0.11	ns	0.09	0.02	0.17	0.02
Height	0.10	-10.26	10.46	ns	6.25	-3.32	15.83	ns	9.10	-3.76	21.96	ns
< 29 yrs[3]												
<39 yrs	-0.08	-1.94	1.78	ns	0.85	-0.95	2.64	ns	-1.83	-4.22	0.56	ns
<49 yrs	-1.60	-3.29	0.08	ns	-1.29	-2.93	0.35	ns	-2.82	-4.99	-0.65	0.01
<59 yrs	-0.22	-2.25	1.82	ns	-0.08	-2.06	1.90	ns	-1.39	-4.01	1.24	ns
60+ yrs	-1.27	-3.71	1.17	ns	-0.41	-2.84	2.03	ns	-4.97	-8.17	-1.78	<0.001
European												
Malay	0.02	-2.21	2.25	ns	-1.20	-3.31	0.92	ns	-7.21	-9.99	-4.43	<0.001
Chinese	-0.65	-2.76	1.46	ns	-1.47	-3.53	0.59	ns	-6.97	-9.67	-4.26	<0.001
Indian	0.22	-1.72	2.16	ns	-0.64	-2.57	1.30	ns	-4.09	-6.59	-1.60	<0.001
AfroCar	5.19	3.35	7.03	<0.001	-0.34	-2.14	1.46	ns	-2.34	-4.75	0.07	ns
p	p<0.0001				ns				p <0.0001			

Table 3.8 (cont) Multiple regression tables for simple linear RV measurements made in the apical view

[3] Age group sample size: RVD-1 ≤ 29 yrs n 47; ≤ 39 yrs n 36; ≤ 49 yrs n 64; ≤ 59 yrs n 30; 60+ n 17. RVD-2 ≤ 29 yrs n 48; ≤ 39 yrs n 42; ≤ 49 yrs n 75; ≤ 59 yrs n 33; 60+ n 18. RVD-3 ≤ 29 yrs n 49; ≤ 39 yrs n 38; ≤ 49 yrs n 70; ≤ 59 yrs n 31; 60+ n 17.

	RVD-AN 46.6%			
	b	95% CI		*p*
Female				
Male	0.94	-0.46	2.34	ns
Mass	0.04	-0.01	0.09	ns
Height	8.16	-0.70	17.02	ns
≤ 29 yrs[4]				
≤39 yrs	0.26	-1.43	1.94	ns
≤49 yrs	0.36	-1.16	1.88	ns
≤59 yrs	1.34	-0.50	3.19	ns
60+ yrs	-0.49	-2.70	1.71	ns
European				
Malay	-2.22	-4.16	-0.29	0.03
Chinese	-3.94	-5.83	-2.06	<0.001
Indian	-2.54	-4.30	-0.78	0.01
AfroCar	4.15	2.45	5.85	<0.001
p	p <0.0001			

Table 3.9 Multiple regression results table from RV area measurements

RVEDA 53.6%	RVESA 48.8

[4] Age group sample size: RVD-AN ≤ 29 yrs n 49; ≤ 39 yrs n 38; ≤ 49 yrs n 74; ≤ 59 yrs n 32; 60+ n 18.

	b	95% CI		p	b	95% CI		p
Female	Reference							
Male	0.97	-0.24	2.17	ns	0.65	-0.12	1.42	0.10
Body Mass	0.05	0.01	0.10	0.02	0.03	0.00	0.06	0.03
Height	9.31	1.59	17.03	0.02	6.88	1.92	11.84	0.01
< 29 yrs[5]	Reference							
<39 yrs	-0.39	-1.80	1.02	ns	-0.07	-0.98	0.84	0.88
<49 yrs	-1.69	-2.98	-0.39	0.01	-0.63	-1.46	0.20	0.14
<59 yrs	-1.53	-3.12	0.05	ns	-0.41	-1.43	0.61	0.43
60+ yrs	-0.80	-2.89	1.29	ns	-0.72	-2.06	0.63	0.29
European	Reference							
Malay	-2.70	-4.41	-1.00	<0.001	-1.16	-2.26	-0.07	0.04
Chinese	-3.16	-4.86	-1.46	<0.001	-1.30	-2.39	-0.21	0.02
Indian	-2.04	-3.54	-0.54	0.01	-0.90	-1.87	0.06	0.07
AfroCar	2.36	0.95	3.77	<0.001	1.62	0.71	2.53	0.00
p	p <0. 0001				p <0.0001			

[5] Age group sample size: RVEDA ≤ 29 yrs n 46; ≤ 39 yrs n 38; ≤ 49 yrs n 66; ≤ 59 yrs n 29; 60+ n 14. RVESA ≤ 29 yrs n 46; ≤ 39 yrs n 38; ≤ 49 yrs n 66; ≤ 59 yrs n 29; 60+ n 14.

3.5. Ratio-scaled RV linear and area dimensions

RV measurements had a weak to modest but nonetheless significant correlation to BSA (r=0.39 – 0.56; p = <0.001) with only RV-WT displaying r values <0.30 (p = 0.001). Height displayed slightly weaker but significant correlations overall with RV-WT displaying the lowest (r= 0.17, p = 0.01). This prompted the need to scale both the linear and area RV measurements by these two respective variables. Using a ratio scaling approach, the simple linear data was scaled to height, and BSA. Tables 3.10 and 3.11 display the mean±SD for each of the ten linear and area measurements, across each ethnic group for ratio-scaled height and BSA.

Despite indexing to both height and BSA, there remained differences between ethnic groups, with the exception of RVD-2 indexed to height.

Assessment of gender based differences revealed some interesting results. Ratiometric scaling of RV linear dimensions to height resulted in gender independent measurements (ns) whilst RVEDA and RVESA remained significant. (p =0.01 & <0.001) respectively. BSA failed to replicate this with significant differences according to gender between RVD-3 (p <0.001), RVOT-2 & 3 (p <0.01), RV-WT (p <0.001) and RVESA (p <0.05).

In addition, despite indexing to common scaling variables, bivariate Pearson's correlations (shown in Table 3.12) still showed significant correlations between RV measurement and either BSA or height, indicating a failure to achieve body size independence. This negated the need for further regression assessment. Despite height showing less linear associations than BSA, both were scaled using allometric principles.

Table 3.10 Indexed (Ratiometric) RV measurements to height (m)

Measurement	Chinese	Malay	Indian	European	Afro-Caribbean	*P*
RVOT-1 (mm/m)	17 ± 2.31	17 ± 2.12	16 ± 2.22	19 ± 2.55	18 ± 2.44	<0.001
RVOT-2 (mm/m)	13 ± 2.13	13 ± 2.08	12 ± 1.53	12 ± 1.67	14 ± 1.67	0.002
RVOT-3 (mm/m)	15 ± 2.07	15 ± 1.8	14 ± 1.76	17 ± 1.91	16 ± 1.99	<0.001
RV-WT (mm/m)	3 ± 0.45	2 ± 0.45	2 ± 0.41	3 ± 0.44	3 ± 0.45	0.001
RVD-1 (mm/m)	18 ± 2.15	19 ± 1.95	18 ± 2.1	18 ± 2.1	22 ± 3.39	<0.001
RVD-2 (mm/m)	18 ± 2.94	18 ± 2.29	17 ± 2.65	18 ± 2.59	18 ± 1.78	ns
RVD-3 (mm/m)	44 ± 3.22	43 ± 2.74	43 ± 3.04	46 ± 3.22	45 ± 4.15	<0.001
RVD-AN (mm/m)	14 ± 2.26	14 ± 2.45	13 ± 1.81	15 ± 1.85	18 ± 2.66	<0.001
RVEDA (cm^2/m)	9 ± 1.62	9 ± 1.5	8 ± 1.46	11 ± 2.07	12 ± 2.4	<0.001
RVESA (cm^2/m)	5 ± 1.12	5 ± 1.13	4 ± 0.96	6 ± 1.23	7 ± 1.55	<0.001

Table 3.11 Indexed (Ratiometric) RV measurements to BSA (mm/m2)

Measurement	Chinese	Malay	Indian	European	Afro-Caribbean	*P*
RVOT-1 (mm/m^2)	16 ± 2.29	15 ± 2.38	15 ± 2.39	17 ± 2.28	16 ± 2.36	<0.001
RVOT-2 (mm/m^2)	12 ± 2.19	12 ± 1.84	11 ± 1.42	11 ± 1.66	12 ± 1.53	0.02
RVOT-3 (mm/m^2)	14 ± 2.16	14 ± 1.68	14 ± 1.73	15 ± 1.99	14 ± 1.73	0.001
RV-WT (mm/m^2)	2 ± 0.45	2 ± 0.43	2 ± 0.45	2 ± 0.40	2 ± 0.42	0.003
RVD-1 (mm/m^2)	18 ± 2.52	18 ± 1.93	17 ± 2.11	17 ± 2.04	19 ± 3.26	<0.001
RVD-2 (mm/m^2)	17 ± 2.96	17 ± 2.27	17 ± 2.91	17 ± 2.63	14 ± 3.11	<0.001
RVD-3 (mm/m^2)	43 ± 4.37	40 ± 4.15	40 ± 3.93	43 ± 4.20	41 ± 4.87	0.001
RVD-AN (mm/m^2)	13 ± 2.44	13 ± 2.47	13 ± 2.07	14 ± 1.87	16 ± 2.35	<0.001
RVEDA (cm^2/m^2)	9 ± 1.62	8 ± 1.71	8 ± 1.61	10 ± 1.87	11 ± 1.95	<0.001
RVESA (cm^2/m^2)	4 ± 1.03	4 ± 1.18	4 ± 1.02	5 ± 1.15	6 ± 1.26	<0.001

Table 3.12 Pre and post ratiometric scaling bivariate Pearson correlations

	Height				BSA			
Dimension	Pre	p	Post	p	Pre	p	Post	p
RVOT-1	0.35	<0.001	-0.04	0.53	0.43	<0.001	-0.59	<0.001
RVOT-2	0.21	<0.001	-0.18	0.01	0.4	<0.001	-0.56	<0.001
RVOT-3	0.38	<0.001	-0.03	0.69	0.53	<0.001	-0.61	<0.001
RV-WT	0.17	<0.01	-0.15	0.02	0.25	<0.001	-0.54	<0.001
RVD-1	0.38	<0.001	0.03	0.69	0.46	<0.001	-0.56	<0.001
RVD-2	0.39	<0.001	0	0.98	0.39	<0.001	-0.62	<0.001
RVD-3	0.51	<0.001	-0.12	0.08	0.48	<0.001	-0.81	<0.001
RVD-AN	0.43	<0.001	0.15	0.03	0.46	<0.001	-0.44	<0.001
RV-EDA	0.55	<0.001	0.38	<0.001	0.55	<0.001	-0.3	<0.001
RV-ESA	0.56	<0.001	0.42	<0.001	0.56	<0.001	-0.23	<0.001

3.6. Allometric Scaled Data

Simple RV measurements were assessed to ascertain if they conformed to TSC and were therefore suitable for allometric scaling. Results (shown in Table 3.13) indicate that height demonstrates a partial agreement to TSC whilst BSA displays no associated agreement. As a result, all data was subsequently scaled for both height and BSA using the allometric process previously described.

Size exponents, beta values (b), (shown in Table 3.14) were calculated for each RV measurement using height and BSA independently. Calculated beta values for height demonstrated 95% confidence intervals traversing 1.0 indicating a close association to a height/RV dimension linear relationship. As a result, only BSA results were scaled allometrically.

Tables 3.15 – 3.18 show the results of the regression performed on the RV dimensions indexed to height. These displayed independence in relation to gender for linear dimensions and EDA. Both age and ethnicity remained significant across a number of measurements. Results in Table 3.15 indicate that with parasternal images RVOT 1-3, increasing age is associated with increasing RV dimensions compared to the $\leq$29 years group. This trend was not replicated in the apical measurements, which displayed no associated age related changes.

Ethnicity displayed various significant relationships compared to the European group. Indian, Chinese and Malay participants displayed significantly small RV dimensions after being indexed to height. The Afro-Caribbean group remained larger after accounting for other cofounding variables in a number of measurements with the exception of RVOT-1, in which it remained significantly smaller (b -1.45 p=0.01).

Simple RV data was scaled using the calculated b values for BSA. The resulting bivariate correlations demonstrated independence from BSA, resulting in no statistical significance (p ns). ANOVA analysis of the BSA scaled data revealed that ethnicity displayed various significant relationships between the five groups ($p < 0.001$) with

European and Afro-Caribbean results exceeding that of Indian Chinese and Malay in various permutations.

Scaling of results to BSA (exp) demonstrated independence in relation to gender with significant ethnic associations remaining. The mean+SD organised by ethnicity are shown in Table 3.18 with the 95% lower and upper reference ranges calculated per ethnic group for comparison. RVD-3 demonstrated the greatest difference between Malay and European upper reference values with +8mm.

Regression analysis (shown in Tables 3.19 – 3.21) demonstrated varying age associated significant results, found in all parasternal measurements (RVOT 1-3 and RV-WT) and RVD-3 ($\leq$49 and 60+ years $p < 0.04$) compared to the $\leq$29 years reference group. Ethnicity remained significant across varying permutations with European measurements still exceeding that of Indian, Chinese and Malay. Despite controlling for other variables, Afro-Caribbean results remained significantly larger across a number of measurements after allometric scaling to BSA.

3.7. Association to RV peak systolic pressure

RV dimensions were correlated with calculated peak systolic pulmonary artery pressure (PSPAP) using all derived raw, scaled and allometrically scaled data sets. This indicated no significant correlations throughout.

Table 3.13 TSC agreement. Results in bold indicate agreement of < 0.05 and are therefore considered suitable for simple ratio scaling.

Variable	BSA			HT		
	TSC		x/y correlation	TSC		x/y correlation
RVOT-1 (mm)	0.79	:	0.43***	**0.37**	**:**	**0.35**
RVOT-2 (mm)	0.56	:	0.31***	0.37	:	0.23*
RVOT3 (mm)	0.59	:	0.31***	**0.39**	**:**	**0.38**
RV-WT (mm)	0.69	:	0.14***	0.31	:	0.17*
RVD-1 (mm)	0.74	:	0.48***	**0.36**	**:**	**0.38**
RVD-2 (mm)	0.69	:	0.22***	**0.38**	**:**	**0.38**
RVD-3 (mm)	1.24	:	0.46***	0.61	:	0.51
RVD-AN (mm)	0.59	:	0.48	0.28	:	0.43*
RVEDA (cm2)	0.45	:	0.55	0.21	:	0.55***
RVESA (cm2)	0.38	:	0.56***	0.18	:	0.56***

*** significant at <0.001, ** significant at <0.01, * significant at <0.05

Table 3.14 calculated beta±SE (95% CI) values for BSA and Height

Variable	BSA (beta±SE (95%CI))				HT (beta±SE (95%CI))			
RVOT-1 (mm)	0.55	±	0.75	0.390 - 0.686	0.94	±	0.164	0.61 - 1.26
RVOT-2 (mm)	0.55	±	0.79	0.388 - 0.699	0.61	±	0.173	0.255 - 0.938
RVOT-3 (mm)	0.63	±	0.066	0.500 - 0.760	0.95	±	0.152	0.657 - 1.256
RV-WT (mm)	0.36	±	0.094	0.170 - 0.542	0.51	±	0.201	0.120 - 0.913
RVD-1 (mm)	0.67	±	0.09	0.454 - 0.800	1.05	±	0.188	0.687 - 1.428
RVD-2 (mm)	0.33	±	0.8	0.332 - 0.646	0.98	±	0.162	0.662 - 1.302
RVD-3 (mm)	0.38	±	0.49	0.285 - 0.479	0.83	±	0.099	0.640 - 1.030
RVD-AN (mm)	0.84	±	0.108	0.602 - 1.028	1.52	±	0.225	1.088 - 1.974
RVEDA (cm2)	1.37	±	0.145	1.081 - 1.654	2.72	±	0.292	2.152 - 3.303
RVESA (cm2)	1.61	±	0.168	1.286 - 1.948	3.14	±	0.34	2.55 - 3.89

Table 3.15 Regression tables for scaled (to height) RV parasternal linear measurements

	RVOT-1 18.2%				RVOT-2 16.2%				RVOT-3 24.8%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female												
Male	0.21	-0.46	0.87	ns	-0.23	-0.78	0.32	ns	-0.34	-0.87	0.18	ns
Body mass	0.02	-0.01	0.04	ns	0.03	0.00	0.05	0.02	0.04	0.02	0.06	<0.001
< 29 yrs												
<39 yrs	0.66	-0.30	1.63	ns	0.50	-0.27	1.27	ns	0.66	-0.11	1.43	ns
<49 yrs	1.38	0.52	2.24	<0.001	1.27	0.57	1.97	<0.001	1.17	0.50	1.85	<0.001
<59 yrs	1.13	0.05	2.20	0.04	1.01	0.16	1.86	0.02	1.08	0.24	1.92	0.01
60+ yrs	0.99	-0.27	2.26	ns	1.27	0.25	2.28	0.02	1.46	0.45	2.47	0.01
European												
Malay	-3.11	-4.19	-2.04	<0.001	-0.25	-1.14	0.65	ns	-2.26	-3.09	-1.42	<0.001
Chinese	-2.75	-3.82	-1.68	<0.001	0.24	-0.65	1.14	ns	-1.96	-2.81	-1.11	<0.001
Indian	-2.29	-3.31	-1.26	<0.001	0.37	-0.48	1.22	ns	-1.70	-2.50	-0.90	<0.001
AfroCar	-1.45	-2.49	-0.42	0.01	1.15	0.30	2.00	0.01	-0.68	-1.48	0.12	ns
p	p <0.0001				p 0.0001				p <0.0001			

Table 3.15 (cont) Regression tables for scaled (to height) RV parasternal linear measurements

	RV-WT 15.9%			
	b	95% CI		p
Female				
Male	-0.13	-0.25	-0.01	0.05
Body mass	0.00	0.00	0.01	ns
< 29 yrs				
<39 yrs	0.28	0.10	0.46	<0.001
<49 yrs	0.18	0.02	0.34	0.03
<59 yrs	0.19	-0.01	0.38	ns
60+ yrs	0.44	0.20	0.67	<0.001
European				
Malay	-0.28	-0.48	-0.09	0.01
Chinese	-0.21	-0.41	-0.02	0.03
Indian	-0.03	-0.22	0.15	ns
AfroCar	0.08	-0.11	0.26	ns
p	p <0.0001			

Table 3.16 Regression table for scaled (to height) RV apical linear measurements

	RVD-1 26.9%				RVD-2 5.1%				RVD-3 16.3%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female												
Male	0.10	-0.69	0.89	ns	-0.01	-0.76	0.75	ns	-0.44	-1.49	0.61	ns
Body mass	0.02	-0.01	0.05	ns	0.01	-0.02	0.04	ns	0.00	-0.04	0.04	ns
< 29 yrs												
<39 yrs	0.20	-0.92	1.33	ns	0.69	-0.40	1.78	ns	-0.46	-1.96	1.03	ns
<49 yrs	-0.52	-1.52	0.48	ns	-0.50	-1.47	0.47	ns	-0.75	-2.08	0.57	ns
<59 yrs	0.36	-0.85	1.57	ns	0.29	-0.90	1.47	ns	0.23	-1.39	1.85	ns
60+ yrs	-0.16	-1.61	1.30	ns	0.16	-1.29	1.61	ns	-1.75	-3.71	0.22	ns
European												
Malay	-0.06	-1.33	1.21	ns	-0.74	-1.96	0.49	ns	-3.41	-5.09	-1.74	<0.001
Chinese	0.68	-0.63	2.00	ns	-0.28	-1.51	0.95	ns	-3.37	-5.06	-1.67	<0.001
Indian	0.51	-0.66	1.68	ns	-0.12	-1.28	1.03	ns	-1.65	-3.22	-0.09	0.04
AfroCar	3.25	2.12	4.37	<0.001	-0.10	-1.19	0.99	ns	-1.06	-2.58	0.46	ns
p	p <0.0001				p ns				p <0.0001			

Table 3.16 (cont) Regression table for scaled (to height) RV apical linear measurements

	RVD-AN 38.4%			
	b	95% CI		*p*
Female				
Male	0.24	-0.46	0.94	ns
Mass	0.01	-0.02	0.04	ns
≤ 29 yrs				
≤39 yrs	0.20	-0.81	1.20	ns
≤49 yrs	0.39	-0.49	1.28	ns
≤59 yrs	0.95	-0.14	2.04	ns
60+ yrs	-0.09	-1.38	1.20	ns
European				
Malay	-2.23	-3.34	-1.11	<0.001
Chinese	-1.20	-2.32	-0.07	0.04
Indian	-1.38	-2.43	-0.33	0.01
AfroCar	2.53	1.51	3.55	<0.001
p	p=<0.0001			

Table 3.17 scaled (to height) RV area measurements

	RVEDA 46.5%				RVESA 41.6%			
	b	95% CI		p	b	95% CI		p
Female								
Male	0.56	-0.02	1.14	ns	0.48	0.10	0.86	0.01
Body mass	0.03	0.01	0.05	0.02	0.02	0.01	0.04	0.01
< 29 yrs								
<39 yrs	-0.24	-1.05	0.57	ns	-0.07	-0.60	0.46	ns
<49 yrs	-0.99	-1.72	-0.26	0.01	-0.41	-0.88	0.07	ns
<59 yrs	-0.88	-1.78	0.02	ns	-0.29	-0.87	0.30	ns
60+ yrs	-0.48	-1.65	0.69	ns	-0.55	-1.31	0.21	ns
European								
Malay	-1.93	-2.90	-0.96	<0.001	-0.81	-1.44	-0.18	0.01
Chinese	-1.50	-2.46	-0.54	<0.001	-0.77	-1.39	-0.14	0.02
Indian	-1.19	-2.05	-0.32	0.01	-0.55	-1.11	0.01	ns
AfroCar	1.44	0.62	2.26	<0.001	0.95	0.42	1.48	<0.001
p	p <0.0001				p <0.0001			

Table 3.18 Mean, standard deviation and 95% reference values for RV dimensions allometrically scaled to BSA (exp).

	RVOT-1mm/m^2(0.55)	RVOT-2 mm/m^2(0.55)	RVOT-3 mm/m^2(0.63)	RV-WT mm/m^2(0.36)	RVEDA cm^2/m2(1.37)
	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)
Afro-Caribbean	21 ± 2.82 16 – 27	16 ± 1.96 Dec-20	18 ± 2.01 14 – 22	4 ± 0.55 3 – 5	9 ± 1.56 6 – 12
European	23 ± 2.87 17 – 28	15 ± 2 11 – 19	19 ± 2.25 15 - 24	3 ± 0.58 3 – 5	8 ± 1.51 5 – 11
Chinese	20 ± 2.53 16 – 25	15 ± 2.43 11 – 20	17 ± 2.27 13 – 22	3 ± 0.55 2 – 5	7 ± 1.45 4 – 10
Indian	19 ± 2.65 14 - 25	15 ± 1.7 11 – 18	17 ± 1.92 13 – 20	3 ± 0.56 2 – 4	6 ± 1.39 4 – 9
Malays	20 ± 2.57 15 - 25	15 ± 2.22 11 – 20	17 ± 1.88 13 – 21	3 ± 0.58 2 – 4	7 ± 1.57 4 – 10
Total	21 ± 2.89 15 - 26	15 ± 2.15 11 – 19	18 ± 2.25 13 - 22	3 ± 0.59 2 – 5	8 ± 1.69 4 - 11
	RVD-1 mm/m^2(0.67)	RVD-2 mm/m^2(0.33)	RVD-3 mm/m^2(0.38)	RVD-AN mm/m^2(0.84)	RVESA cm2/m^2(1.61)
	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)	Mean±SD (95% range)
Afro-Caribbean	24 ± 3.84 16 – 31	23 ± 2.27 18 – 27	60 ± 5.47 49- 71	18 ± 2.53 13 - 23	4 ± 0.85 2 – 6
European	21 ± 2.34 16 - 25	23 ± 3.23 17 – 29	63 ± 4.72 54 – 72	16 ± 1.97 12 - 19	4 ± 0.84 2 – 5
Chinese	21 ± 2.56 16 – 26	23 ± 3.62 16 – 30	59 ± 3.63 52 – 66	15 ± 2.53 10 – 20	3 ± 0.76 2 – 5
Indian	20 ± 2.41 16 – 25	22 ± 3.71 15 – 29	57 ± 3.9 49 - 64	14 ± 2.18 9 - 20	3 ± 0.78 2 – 5
Malays	21 ± 2.1 17 – 25	22 ± 2.74 17 – 27	56 ± 3.73 49 - 64	14 ± 2.61 9 – 20	3 ± 0.94 1 – 5
Total	21 ± 3.04 15 - 27	23 ± 3.16 16 - 29	59 ± 4.84 50 - 69	15 ± 2.77 10 - 21	3 ± 0.9 2 - 5

Table 3.19 Regression tables for allometrically scaled (to BSA) RV parasternal linear measurements

	RVOT-1 15.9%				RVOT-2 6.6%				RVOT-3 16.1%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference											
Male	0.67	-0.05	1.39	ns	0.19	-0.40	0.77	ns	0.02	-0.53	0.57	ns
≤ 29 yrs	Reference											
≤39 yrs	0.46	-0.65	1.57	ns	0.47	-0.43	1.36	ns	0.56	-0.29	1.42	ns
≤49 yrs	1.23	0.23	2.22	0.02	1.22	0.41	2.03	<0.001	1.08	0.32	1.83	0.01
≤59 yrs	1.24	-0.03	2.50	ns	0.99	0.00	1.99	ns	1.04	0.09	1.99	0.03
60+ yrs	0.92	-0.55	2.39	ns	1.25	0.07	2.42	0.04	1.48	0.34	2.61	0.01
European	Reference											
Malay	-3.70	-4.92	-2.47	<0.001	-0.12	-1.15	0.91	ns	-2.72	-3.64	-1.79	<0.001
Chinese	-3.89	-5.14	-2.65	<0.001	-0.46	-1.49	0.57	ns	-2.92	-3.85	-1.99	<0.001
Indian	-2.84	-3.99	-1.68	<0.001	0.28	-0.68	1.24	ns	-2.17	-3.03	-1.31	<0.001
AfroCar	-1.94	-3.14	-0.74	<0.001	1.21	0.22	2.20	0.02	-1.04	-1.95	-0.14	0.02
p	p <0.0001				p 0.006				p <0.0001			

Table 3.19 (cont) Regressiontables for allometrically scaled (to BSA) RV parasternal linear measurements

	RV-WT 11.8%			
	b	95% CI		*p*
Female				
Male	-0.03	-0.18	0.12	ns
≤ 29 yrs				
≤39 yrs	0.31	0.08	0.54	0.01
≤49 yrs	0.18	-0.02	0.38	ns
≤59 yrs	0.23	-0.03	0.48	ns
60+ yrs	0.54	0.24	0.85	<0.001
European				
Malay	-0.39	-0.64	-0.14	<0.001
Chinese	-0.44	-0.69	-0.19	<0.001
Indian	-0.12	-0.36	0.11	ns
AfroCar	0.08	-0.17	0.32	ns
p	p=<0.0001			

Table 3.20 Regression tables for allometrically scaled (to BSA) RV Apical linear measurements

	RVD-1 16.0%				RVD-2 2.4%				RVD-3 22.4%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female												
Male	0.25	-0.57	1.07	ns	0.52	-0.34	1.38	ns	1.05	-0.17	2.27	ns
≤ 29 yrs												
≤39 yrs	-0.05	-1.32	1.21	ns	0.59	-0.77	1.95	ns	-1.63	-3.52	0.26	ns
≤49 yrs	-1.07	-2.20	0.05	ns	-1.11	-2.32	0.09	ns	-2.37	-4.05	-0.69	0.01
≤59 yrs	0.06	-1.33	1.44	ns	-0.09	-1.58	1.39	ns	-0.64	-2.73	1.45	ns
60+ yrs	-0.60	-2.24	1.05	ns	-0.30	-2.12	1.51	ns	-3.80	-6.30	-1.31	<0.001
European												
Malay	0.63	-0.81	2.07	ns	-0.75	-2.24	0.74	ns	-5.30	-7.40	-3.20	<0.001
Chinese	-0.09	-1.50	1.33	ns	-0.94	-2.44	0.56	ns	-5.36	-7.47	-3.25	<0.001
Indian	0.72	-0.55	1.99	ns	-0.25	-1.63	1.13	ns	-2.75	-4.66	-0.83	0.01
AfroCar	3.19	1.92	4.46	<0.001	-0.38	-1.75	0.98	ns	-2.22	-4.16	-0.29	0.03
p	p <0.0001				p ns				p <0.0001			

Table 3.20 (cont) Regression tables for allometrically scaled (to BSA) RV Apical linear measurements

	RVD-AN 25.4%			
	b	95% CI		*p*
Female		Reference		
Male	0.03	-0.65	0.71	ns
≤ 29 yrs		Reference		
≤39 yrs	-0.13	-1.19	0.93	ns
≤49 yrs	0.03	-0.91	0.96	ns
≤59 yrs	0.98	-0.19	2.15	ns
60+ yrs	-0.32	-1.69	1.05	ns
European		Reference		
Malay	-1.02	-2.19	0.14	ns
Chinese	-2.20	-3.37	-1.03	<0.001
Indian	-1.16	-2.23	-0.09	0.03
AfroCar	2.17	1.09	3.26	<0.001
p	p=<0.0001			

Table 3.21 Regression tables for allometrically scaled (to BSA) RV apical area measurements

	RVEDA 23.1%				RVESA 14.0%			
	b	95% CI		p	b	95% CI		p
Female	Reference							
Male	-0.01	-0.45	0.42	ns	0.07	-0.18	0.31	ns
≤ 29 yrs	Reference							
≤39 yrs	-0.33	-1.00	0.34	ns	-0.10	-0.47	0.28	ns
≤49 yrs	-0.88	-1.48	-0.28	<0.001	-0.30	-0.64	0.03	ns
≤59 yrs	-0.81	-1.56	-0.06	0.03	-0.23	-0.66	0.19	ns
60+ yrs	-0.31	-1.28	0.65	ns	-0.33	-0.87	0.21	ns
European	Reference							
Malay	-0.84	-1.61	-0.07	0.03	-0.30	-0.73	0.13	ns
Chinese	-1.10	-1.89	-0.31	0.01	-0.36	-0.81	0.08	ns
Indian	-0.58	-1.27	0.11	ns	-0.23	-0.62	0.16	ns
AfroCar	0.89	0.21	1.57	0.01	0.49	0.11	0.87	<0.01
P	p <0.0001				p <0.0001			

To further understand the impact that ethnicity, independent of body size, specifically had on these measurements, further covariate analysis was undertaken using the model $y = a{:}x^{b}.\exp(c.\text{ethnicity})$.

Results for BSA and height, shown in Table 3.19 revealed that despite producing significant results within the regression analysis, ethnicity contributed very little to the actual model. This was expressed in the small c values displayed in addition to the relatively small change in b values found in Table 3.22. This indicates that although ethnicity remained significant in varying degrees across the regression modelling, it is unlikely to be related to body size. Scaled results using newly derived b values did not result in significantly altered outcomes (ns) when compared to scaled data shown in Table 3.18.

Table 3.22 scaling exponents for BSA and ethnicity.

	c (height)	c (BSA)
RVOT-1	0.02	0.01
RVOT-2	0.01	0.007
RVOT-3	0.02	0.05
RV-WT	0.01	0.01
RVD-1	0.02	0.02
RVD-2	0.01	0
RVD-3	0.01	0.01
RVD-AN	0.04	0.04
RVEDA	0.05	0.05
RVESA	0.05	0.05

3.8. 2D Repeatability

Repeat analysis results are presented according to the acquisition window. This creates a logical grouping of the measurements compared across each of the three test scenarios (intraobserver, interobserver and retest). The full results can be seen in Table 3.23.

3.8.1. Parasternal Short Axis Window (PSAX)

PSAX images RVOT-1 and RVOT-2 showed no systematic bias. All ICC scores were either good or excellent with intraobserver RVOT-1 displaying the highest score of 0.85 and the RVOT-2 retest displaying the lowest (ICC 0.56). The COV was highest for interobserver RVOT-2 (COV 14%) but collectively higher in the retest group (COV 13% and 12% RVOT-1 and RVOT-2) respectively. The lowest score was found with RVOT-1 in the intraobserver group. The LOA were narrow though progressively increased for each measurement across the three test scenarios.

3.8.2. Parasternal Long Axis Window (PLAX)

No systematic bias was demonstrated except for both inter and intraobserver RVOT-3 ($p<0.05$). RVOT-3 ICC scores ranged from good to very good whilst RV WT ranged from moderate for both interobserver and retest (ICC 0.34 and 0.50 respectively) to good (ICC 0.64). COV for RVOT-3 interobserver was highest (COV 13%) but remained similar to both the retest (12%) and intraobserver (10%) values. RV wall thickness displayed a larger COV for both intraobserver (19%) and interobserver (19%) with a lower variation noted in the retest group (13%). Wider, one-sided RVOT-3 LOA were found with both intraobserver and interobserver agreement while narrow LOA were found for all RV wall thickness measurements.

3.8.3. Apical Four Chamber (A4C)

No intraobserver or interobserver systematic bias was found except for interobserver RVD-3 and RVEDA ($p<0.05$). Retest analysis showed significant ANOVA results for RVD-1 and RVESA ($p<0.05$). Intraobserver ICC scores ranged from moderate for RVD-2 (ICC 0.53) to very good for RVD-1 and RVD-3 (ICC 0.89 and 0.91 respectively).

Interobserver ICC ranged from poor for RVD-2 (ICC 0.31) to excellent for RVD-3 and RVD-1 (ICC 0.86 & 0.87 respectively). Apical retest ICC scores ranged from poor for RVD-3 (ICC 0.19) to good to excellent for RVD-AN, RVD-1, RVEDA and RVESA (ICC scores 0.64, 0.71, 0.78 and 0.75) respectively. The COV scores ranged from 5% to 21 % for intraobserver and 5% to 24% for interobserver for both RVD-3 and RVESA.

Retest scores were cumulatively higher with the lowest (RVD-1) at 11% and highest (RVD-3) at 20%. RVD-2 showed the widest interobserver and intraobserver LOA. The remaining measurements from both observations showed narrow LOA in comparison to the retest scenario. Both end diastolic and end systolic LOA were similar for both intraobserver and interobserver analysis. Retest results displayed wider LOA, however.

Table 3.23 Repeatability statistics organised by echocardiographic window.

	Window	PSAX		PLAX		A4C					
	Variable	RVOT 1	RVOT 2	RVOT 3	RV WT	RVD 1	RVD 2	RVD 3	RVD-AN	RVEDA	RVESA
Intra Observer	COV (%)	6	9	10*	19	7	15	5	10	14	21
	ICC	0.85	0.75	0.85	0.64	0.89	0.53	0.91	0.79	0.63	0.71
	95% LOA (mm)	-2.2 - 4.4	-2.9 - 4.1	-2.7 - 7.3	-1.6 - 1.3	-5.6 - 3.9	-9.4 - 8.5	-6.1 - 6.9	-4.1 - 4.2	-4.7 - 5.1	-3.5 - 3.8
Inter Observer	COV (%)	8	14	12*	19	7	21*	5*	10	16*	24
	ICC	0.78	0.60	0.66	0.34	0.87	0.31	0.86	0.77	0.78	0.60
	95% LOA (mm)	-3.2 - 5.9	-4.7 - 5.8	-3.6 - 8.5	-1.3 - 1.5	-4.1 - 5.6	-6.2 - 15	-5.8 - 9.0	-4.6 - 3.9	-6.8 - 4.6	-3.5 - 4.8
Test Retest (Intra Observer)	COV (%)	13	12	12	13	11*	16	20	13	15	17*
	ICC	0.66	0.56	0.66	0.50	0.71	0.37	0.19	0.64	0.78	0.75
	95% CI	0.40 - 0.82	0.26 - 0.76	0.39 - 0.82	0.17 - 0.72	0.43 - 0.85	0.02 - 0.64	-0.19 - 0.51	0.38 - 0.81	0.59 - 0.89	0.50 - 0.88
	95% LOA (mm)	-7.5 - 8.3	-6.2 - 4.9	-6.9 - 6.5	-1.2 - 1.1	-10.3 - 6.3	-8.4 - 10.9	-15.9 - 8.6	-9.0 - 7.1	-6.2 - 6.9	-2.8 - 4.7

* -denotes $p<0.05$ from ANOVA

3.9. Speckle Tracking Results

The mean age of enrolled subjects was 40 +11 years (age range 19 – 73 years) 55% were male. Significant differences in age between ethnic groups were found with the European groups the youngest ($p<0.001$). Age did not differ significantly between male or female groups.

Acquisition of strain data was similar between Chinese (77%), European (76%), Afro-Caribbean (75%) and Indian (74%) groups. The Malay group showed the lowest with 52% acquirement. When assessed by age group, no significant differences in terms of gender or heart rate were found between the decades. The mean study frame rate was 76±18 fps with a mean tracking quality score of 1.0±0.08. Table 3.24 shows the clinical characteristics for each ethnic group.

Compared to the linear dimensions cohort, the STE volunteers were on average of similar height (166±9cm vs. 166±12cm) but younger (40±11yrs vs. 43±11yrs) with a lower body mass (69±13kg vs. 71.3±14kg). LV internal dimensions (LVID) were also similar, IVSd 0.78±0.14 vs. 0.80±0.15cm, LVIDd 4.70±0.46cm vs. 4.66±0.48cm, LVPWd 0.75±0.13cm vs. 0.76±0.14cm and LVIDs 2.89±0.45cm vs. 2.85±0.47cm. The ejection fractions were also similar compared to the 2D linear data (63.45±5.22% vs. 63.53±5.33%).

Table 3.24 Clinical characteristics and LV measurements from the strain cohort, for each ethnic group

	Afro-Caribbean (n=45)	Chinese (n=51)	European (n=35)	Indian (n=34)	Malay (n=25)
Male (%)	53	49	57	62	60
Mean age (years)	37 ± 8	44 ± 11	32 ± 11	44 ± 10	42 ± 12
HR (bpm)	66 ± 10.4	69 ± 12.3	65 ± 12.7	69 ± 9.4	71 ± 15.9
Height (cm)	170 ± 8.0	163 ± 10.3	172 ± 10.9	163 ± 8.3	163 ± 7.9
Body Mass (Kg)	76 ± 13.0	63 ± 12.0	73 ± 13.5	67 ± 12.9	68 ± 9.6
IVSd (mm)	8 ± 1.5	8 ± 1.5	8 ± 1.4	8 ± 1.3	8 ± 1.5
LVIDd (mm)	47 ± 3.4	47 ± 4.9	49 ± 5.2	45 ± 4.5	47 ± 4.7
PWd (mm)	8 ± 1.3	8 ± 1.4	8 ± 1.3	8 ± 1.4	8 ± 1.2
LVIDs (mm)	29 ± 3.9	29 ± 4.3	32 ± 4.4	27 ± 5.2	29 ± 4.1
EF(%)	63 ± 4.9	64 ± 5.3	63 ± 5.2	68 ± 5.7	63 ± 5.2
TAPSE (cm)	2.4 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.2 ± 0.2	2.4 ± 0.3
TV E (m/s)	0.50 ± 0.11	0.52 ± 0.10	0.55 ± 0.10	0.54 ± 0.09	0.53 ± 0.09
TV A (m/s)	0.33 ± 0.10	0.34 ± 0.07	0.31 ± 0.07	0.35 ± 0.09	0.35 ± 0.10
TVDec (ms)	173 ± 48	217 ± 51	210 ± 65	192 ± 53	185 ± 55
S'Prime (m/s)	13 ± 2.0	13 ± 1.7	13 ± 1.8	13 ± 1.9	13 ± 2.2
FAC (%)	47 ± 5.7	49 ± 7.9	48 ± 7.0	46 ± 8.1	48 ± 7.6

Results were organised as either global or RV freewall strain. Comparisons between global strain values and RV freewall mean strain values, shown in Table 3.24, displayed highly significant differences ($p < 0.001$), with the RV freewall displaying increased levels of PSS, PSSR, SRe and SRa. Figure 3.3 presents significantly higher

(more negative) levels of strain and strain rate within the RV freewall compared to the global measurements organised by age group, with the exception of PSS within the 60+ age group.

Bivariate Pearson correlations show a significant but weak level of association between RV Freewall PSS and TAPSE (r = -0.194 p =0.009) and RV TDi (r =-0.16 p =0.03). There was no significant correlation with FAC (r = 0.11 p ns). Similar results were found for global analysis with a significant but weak level of correlation between global peak systolic strain (GPSS) and TAPSE (r = -0.24 p <0.001) and RV TDi (r =-0.24 p = 0.002). There was no significant correlation with FAC (r = 0.11 p ns).

Women demonstrated increased GPSS (-24.4%±3.5% versus -22.6%±3.4% p <0.01) global peak systolic strain rate (GPSSR) (-1.3±0.21/s versus -1.2±0.21/s p <0.05) and early global diastolic strain rate (GDSRe) (1.5±0.41/s versus 1.3±0.31/s p <0.0001) compared to males. RV freewall results were similar, with women demonstrating increased PSS (-28.36±5.0% versus -26.57±4.6% p <0.01) PSR (-1.96±0.421/s versus -1.79±0.451/s p <0.01) and SRe (2.16±0.521/s versus 1.79±0.471/s p =<0.0001). Both global late diastolic strain rate (GDSRa) and RV freewall SRa were not significant.

Table 3.25 Collective strain and SR data for both global and RV freewall measurement techniques.

	Global		RV freewall		p
Peak systolic strain (%)	-23	± 3.6	-27	± 4.9	<0.001
Peak systolic SR (1/s)	-1.25	± 0.2	-1.87	± 0.5	<0.001
Diastolic SRe (1/s)	1.38	± 0.4	1.96	± 0.5	<0.001
Diastolic SRa (1/s)	1.05	± 0.3	1.40	± 0.4	<0.001

Multiple regression modelling shown in Tables 3.26 for the RV freewall indicated that both gender (b= 2.37 p =0.02) and body mass (b= 0.09 p =0.02) were significant

predictors for PSS, with males displaying reduced (i.e. less negative) levels of longitudinal function.

After controlling for age, gender, anthropometric and heart rate data, the Afro-Caribbean population showed lower levels of RV freewall SR (b=0.21, p=0.04) compared to the European reference population. Within the same regression model, both significant contributions from heart rate (b= -0.01, p <0.001) and body mass (b =0.01, p <0.01) were found.

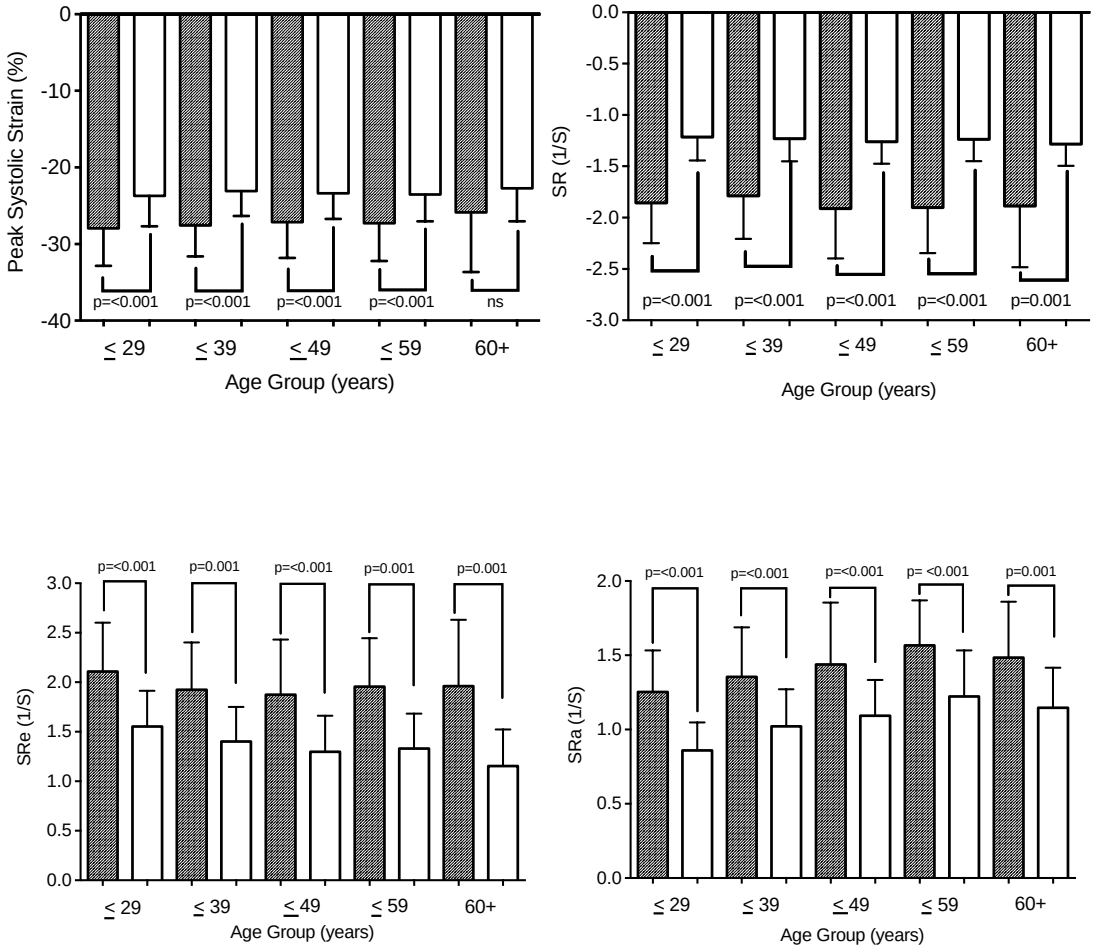

Figure 3.3 Global (open bar) versus freewall (closed bar) strain and strain rate results, organised by age groups. Distribution across age groups was as follows: ≤ 29 yrs *n* 47, ≤39 yrs *n* 38, ≤49 yrs *n* 65, ≤ 59 yrs and 60+ yrs *n* 13. Total group *n* 190.

RV freewall SRe demonstrated significant results for gender (b =-0.34, p <0.001), and body mass (b =-0.01, p <0.001) but no significant age related changes. Late diastolic SRa showed an increase with advancing age in the < 49 yrs (b =0.21, p =0.01) and < 59 yrs (b =0.34, p <0.001). The Afro-Caribbean group showed reduced late diastolic

SR (b =-0.18, p =0.03) After controlling for all other covariates; gender was not significant in the model.

In comparison, regression analysis using the six-segment global method, shown in Table 3.27, revealed no ethnic variation across any strain parameters. Gender (b=1.83, P <0.01), body mass (b =0.08, p <0.01) and HR (b=0.05, p =0.03) were identified as independent predictors of GPSS. GPSSR showed no gender bias. HR (b =-0.004, p =0.003), body mass (b =0.004 p =0.01) displayed significant associations.

A significant reduction in GDSRe was noted compared to the youngest age group in the over 40s (b =-0.24, p <0.01 b=-0.25, p =0.003 and b=-0.42, p <0.001 respectively) in addition to body mass (b=-0.01, p <0.001). Age was the sole predictor for GDSRa with each age group showing an increase compared to the $\leq$29 year group (in ascending age group: (b=0.17, p=0.002; b=0.23, p <0.001; b=0.37, p <0.001; b=0.25, p =0.003) respectively.

Table 3.26 RV freewall strain regression results

	RV-freewall PSS(%) $R^2$11.3%				RV-freewall PSSR $R^2$17.6%				RV-freewall SRe R^2 27.7%				RV-freewall SRa R^2 15.6%			
	b	95% CI		p	B	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference															
Male	2.37	0.43	4.31	0.02	0.07	-0.10	0.25	ns	-0.34	-0.53	-0.15	<0.001	-0.06	-0.20	0.09	ns
Height	-0.10	-0.22	0.02	ns	0.00	-0.02	0.01	ns	0.01	0.00	0.02	ns	0.00	-0.01	0.01	ns
Body mass	0.09	0.02	0.17	0.02	0.01	0.00	0.02	0.01	-0.01	-0.02	-0.01	<0.001	0.00	-0.01	0.00	ns
≤29 yrs	Reference															
≤39 yrs	-0.59	-2.78	1.60	ns	-0.06	-0.25	0.14	ns	-0.05	-0.26	0.17	ns	0.16	0.00	0.32	ns
≤49 yrs	0.67	-1.41	2.76	ns	-0.10	-0.29	0.09	ns	-0.21	-0.42	0.00	ns	0.21	0.05	0.36	0.01
≤ 59 yrs	1.07	-1.46	3.59	ns	-0.08	-0.31	0.15	ns	-0.18	-0.43	0.07	ns	0.34	0.16	0.53	<0.001
60+ yrs	2.22	-0.98	5.41	ns	-0.04	-0.33	0.25	ns	-0.16	-0.48	0.16	ns	0.22	-0.02	0.46	ns
European	Reference															
Malay	-0.38	-3.01	2.24	ns	0.06	-0.18	0.29	ns	-0.12	-0.38	0.14	ns	-0.16	-0.35	0.04	ns
Chinese	-0.47	-2.85	1.92	ns	0.15	-0.07	0.37	ns	0.00	-0.24	0.24	ns	-0.05	-0.22	0.13	ns
Indian	-1.24	-3.98	1.50	ns	-0.03	-0.29	0.23	ns	0.22	-0.06	0.50	ns	0.04	-0.17	0.24	ns
AfroCar	0.77	-1.48	3.03	ns	0.21	0.01	0.42	0.04	-0.10	-0.32	0.12	ns	-0.18	-0.35	-0.02	0.03
HR	0.04	-0.02	0.10	ns	-0.01	-0.01	0.00	<0.001	0.00	0.00	0.01	ns	0.00	0.00	0.01	ns
p	p <0.001				P 0.0001				p <0.0001				p <0.004			

Table 3.27 Global RV strain regression tables

	Global PSS (%) R^2 11.9%				Global PSSR R^2 15.5%				Global diastolic SReR^{2}32.1%				Global diastolic SRa R^2 26.2%			
	b	95% CI		p	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference															
Male	1.83	0.47	3.19	0.01	0.04	-0.04	0.13	ns	-0.24	-0.37	-0.11	<0.001	0.03	-0.07	0.13	ns
Height	-0.05	-0.14	0.03	ns	0.00	-0.01	0.00	ns	0.01	0.00	0.01	ns	0.00	-0.01	0.01	ns
Body mass	0.08	0.03	0.13	<0.001	0.00	0.00	0.01	0.01	-0.01	-0.02	-0.01	<0.001	0.00	-0.01	0.00	ns
< 29 yrs	Reference															
<39 yrs	-0.25	-1.79	1.28	ns	-0.07	-0.16	0.03	ns	-0.06	-0.20	0.09	ns	0.17	0.06	0.28	<0.001
<49 yrs	0.32	-1.15	1.77	ns	-0.06	-0.15	0.03	ns	-0.24	-0.38	-0.10	<0.001	0.23	0.13	0.34	<0.001
<59 yrs	0.64	-1.14	2.41	ns	-0.03	-0.14	0.08	ns	-0.25	-0.42	-0.09	<0.001	0.37	0.24	0.50	<0.001
60+ yrs	1.28	-0.96	3.52	ns	-0.06	-0.20	0.08	ns	-0.42	-0.63	-0.21	<0.001	0.25	0.09	0.41	<0.001
European	Reference															
Malay	0.32	-1.52	2.16	ns	-0.02	-0.13	0.10	ns	-0.08	-0.25	0.10	ns	-0.10	-0.23	0.03	ns
Chinese	-0.04	-1.72	1.63	ns	0.02	-0.09	0.12	ns	0.01	-0.16	0.17	ns	0.06	-0.07	0.18	ns
Indian	-0.45	-2.37	1.47	ns	-0.05	-0.16	0.07	ns	0.07	-0.12	0.25	ns	0.01	-0.13	0.15	ns
AfroCar	1.17	-0.41	2.76	ns	0.07	-0.03	0.17	ns	-0.05	-0.20	0.11	ns	-0.02	-0.14	0.09	ns
HR	0.05	0.00	0.09	0.03	0.00	-0.01	0.00	<0.001	0.00	0.00	0.00	ns	0.00	0.00	0.01	ns
p	p <0.001				p 0.003				p <0.0001				p <0.0001			

3.10. Repeatability

RV freewall PSS showed excellent ICC scores for both intraobserver (ICC = 0.86) and interobserver (ICC = 0.86) analysis. With the addition of both a second sonographer and scan, this reduced to a good level of agreement (ICC = 0.56).

RV freewall PSSR ICC scores ranged from excellent (0.78 intraobserver) to good, ICC = 0.58, for interobserver and 0.51 for retest. Early diastolic strain rate ICC scores showed excellent levels of agreement with intraobserver (0.80) and interobserver (0.76) and good agreement for retest analysis (0.42). Late diastolic strain rate showed good agreement for both intraobserver and retest with ICC scores of 0.67 and 0.42 respectively. Good agreement was found with interobserver analysis.

Bland Altman agreement showed no systematic bias ($p>0.05$). Graphs for each of the three test scenarios are shown in Figure 3.4. Similar LOA (~+5%) were demonstrated between PSS test scenarios. Despite this, interobserver analysis displayed wider LOA across both PSR and SRe results.

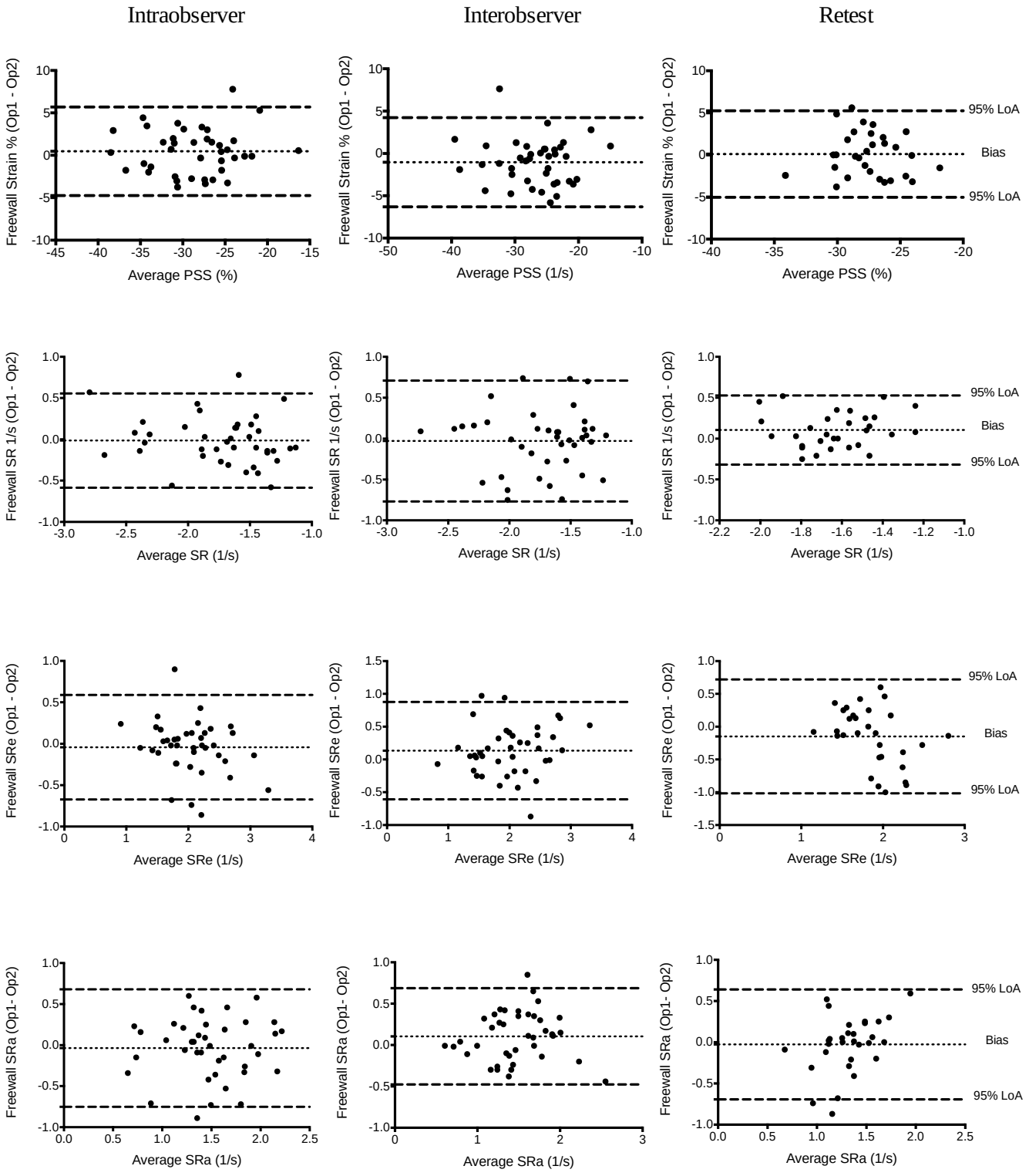

Figure 3.4 Bland Altman graphs displaying agreement for RV-freewall measurements across all three test scenarios.

3.11. Segmental Strain Assessment

Segmental results derived from basal, mid and apical septal and lateral walls organised by gender are shown in Table 3.28. In addition to global and freewall analysis, segmental regression analysis of the longitudinal strain parameters was undertaken. For the purpose of this assessment, segments are referred to as either lateral (RV freewall) or septal. This allowed for a more detailed examination of the particulars that separate global and freewall strain results, while also enabling a better understanding of how clinically meaningful segmental analysis is as a technique.

3.11.1. Peak Systolic Strain

Segmental analysis of PSS results showed that gender had a significant effect on PSS in both septal and lateral basal regions, as well as the apical lateral region ($p < 0.05$) and a highly significant effect in the adjacent apical and mid septal regions ($p < 0.001$) with females displaying increased PSS compared to males.

Both mid-septal and lateral segments displayed a significant difference compared to the reference age group. Interestingly the mid-lateral showed increased levels of longitudinal strain ($p < 0.05$) whilst the mid-septal showed significantly reduced levels ($p < 0.05$).

Despite a higher PSS value being recorded in the apical lateral region ($p < 0.05$) of the European group, compared to the Afro-Caribbean group, the pattern was reversed within the basal lateral segments ($p < 0.05$). This reversed base-to-apex gradient was seen across all four ethnic groups, but was only significant between the European and Afro-Caribbean volunteers.

Despite the significant results displayed on the lateral wall segments, the R^2 value remained non-significant at 7.5%, 6.5% and 5.8% for the apical, mid and basal regions respectively. Both the apical and mid-septal walls were significant (R^2 = 11.2% $p < 0.05$ and 13% $p < 0.01$). Basal lateral R^2 was not significant.

3.11.2. Peak systolic Strain Rate

Strain rate results showed similar segmental, ethnic group variations with both the apical lateral ($p < 0.01$) and apical septal ($p < 0.05$) regions in the European group showing an increased rate of myocardial deformation compared to the Afro-Caribbean group. In addition, the basal septal Malay group also showed an increased rate of deformation compared to the European group ($p < 0.05$).

Gender was significant in the mid-septal ($p < 0.05$), apical lateral ($p < 0.01$) and apical septal ($p < 0.001$) segments. Females typically had an increased rate of myocardial deformation compared to males. Age was not significant in this particular model. R^2 values were significant in the apical lateral (R^2 = 12.1%), apical septal (12.9%) and mid-septal regions (13.1%) for each segment $p < 0.01$.

3.11.3. Early Diastolic Strain Rate

Compared to the European reference group, the Afro-Caribbean group ($p < 0.05$) displayed reduced ‘e’ waves whilst the Malay group, after adjustment for gender and age, displayed significantly higher ‘e’ waves ($p < 0.01$). Gender was significant in all six regions with males showing reduced peak ‘e’ wave results compared to females.

These were highly significant in both apical and mid-septal and lateral walls ($p < 0.001$) and significant in the basal septal and lateral wall ($p < 0.01$). Age tended to show decreasing values across all segments with significant results in the <49 year group in both the apical and basal lateral and the apical and mid-septal regions ($p < 0.05$). The <59 years groups showed one significant reduction in the mid-septal region ($p < 0.05$) whilst the 60+ groups showed a significant reduction in the apical septal region ($p < 0.01$).

All R^2 models were significant bar the basal septal region. Both mid and basal lateral wall were significant at $p < 0.01$, whilst both the apical and the mid-septal region were significant at $p < 0.05$.

3.11.4. Late Diastolic Strain Rate

Significant ethnic differences were seen in the Indian apical lateral and Afro-Caribbean basal lateral ($p < 0.05$) with increased late diastolic a waves compared to the European sample. Highly significant reductions in late diastolic a waves were noted in the mid-septal region in the Chinese and Malay groups and the Chinese basal septal region ($p < 0.01$). Diastolic changes varied across each of the regions.

Gender showed no significant influence on the model. Age group results displayed an increasing trend in diastolic 'a' wave from $\leq$29 years to $\leq$59 years. This trend was highly significant in the $\leq$59 age group ($p < 0.001$) in the apical lateral region and significant in the <49 years and $\leq$59 years mid lateral group ($p < 0.05$). The basal lateral group showed no significant difference with age.

Septal results showed a significant result in the $\leq$39 years, 60+ years apical septal and $\leq$39yrs basal septal group ($p < 0.01$) and highly significant differences in the remaining age groups across all septal regions. Mid-septal age results were all highly significant.

This contribution from age to the model resulted in significant R^2 values in the apical lateral (12.6%) and mid-lateral (9.5%) ($p < 0.05$) and highly significant R^2 values in septal regions, apical septal (20.5%) mid septal (40.4%) and basal septal (25.5%) ($p < 0.001$). All results showed an increasing trend in 'a' waves compared to the $\leq$29yrs group up to $\leq$59 yrs with a significant increasing (compared to the reference value) but a trend reduction in the 60+ age group.

3.12. Segmental Reproducibility

COV showed a large degree of measurement variability. ICC testing demonstrated poor reliability across all four strain variables. 95% confidence intervals transversed zero and therefore could not be clinically interpreted.

Interobserver - PSS displayed LOA of approximately +10% in the apical septal and lateral regions. Both basal and mid-septal segments displayed lower LOA (-5 to 3.5%, -4.4 to 4.1%) compared to lateral regions (-11 to 10%, -7.0 to 8.0%) but similar ICC scores of 0.71, 0.71 and 0.68, 0.73 respectively.

Intraobserver SR ICC scores ranged from good in the apical, basal and mid lateral regions (0.44, 0.42 and 0.41 respectively) to 0.69, 0.72 and 0.62 respectively in the septal regions. COV ranged from 22% to 31% across all six segments. good to excellent agreement using ICC was observed across all diastolic SR measurements with apical septal (SRe) the lowest at 0.43 and mid septal (SRa) the highest at 0.85. COV varied from 19% for basal septal SRa, to 37% for apical septal SRe.

Both agreement and reproducibility scores were cumulatively lower in this group compared to Intraobserver results. ICC scores ranged from <0.1 to 0.43 for PSS. Segmental SR showed low levels of ICC ranging from <0.1 to 0.33. Both early diastolic SR and late diastolic SR displayed poor to good ICC scores at <0.1 to 0.43 and 0.17 to 0.59 respectively. COV scores were low across all strain measurements in all regions, ranging from 20% in the apical lateral region for PSS to 49% in the apical septal region during late diastolic SR (SRa).

LOA typically started from >+10% for PSS, with the exception of the basal septal and mid-septal regions. In addition LOA >1.0 1/s were found with all apical lateral and apical septal measurements.

Single analyser assessment of repeat scans by two operators displayed poor agreement across regional measurements. PSS ICC scores ranged from <0.1 to in the apical regions to 0.38 in the basal septal segment. Regional SR scores displayed similar segmental trends, while apical measurements showed ICC scores of <0.1 the mid-lateral was the highest, however with a good ICC score of 0.44.

Both early and late diastolic SR showed improved ICC results. Apical lateral, septal and mid-septal SRe all recorded good ICC scores (0.44, 0.40 and 0.49 respectively). Basal septal was the lowest with an ICC of 0.10, while apical septal SRa was the highest (ICC 0.51). Conversely apical lateral was lower with an ICC score of 0.21.

COV measurements ranged from 16% in the mid-lateral region to 40% in the apical septal region for PSS. This archetype was followed with PSSR measurements, although increased COV were found (20% and 43% respectively). PSS LOA exceeded 10% in all regions with the exception of the basal septal (-5.9 to 6.3%) and the mid-septal (-8.2 to 9.8). Wide LOA were also noted across both systolic and diastolic SR measurements.

Table 3.28 Mean(+SD) strain results organised by gender

Region	PSS (%)	PSSR (1/s)	SRe (1/s)	SR a (1/s)
Female				
Apical lateral	-26.88 ± 6.67†	-1.74 ± 0.42**	2.19 ± 0.76^	1.51 ± 0.53
Mid lateral	-29.12 ± 5.67	-1.76 ± 0.39	1.96 ± 0.49^	1.31 ± 0.45
Basal lateral	-29.04 ± 7.02	-2.09 ± 0.59	2.35 ± 0.73^	1.54 ± 0.58
Apical septal	-21.43 ± 6.49*	-1.60 ± 0.48***	2.02 ± 0.66^	1.27 ± 0.51
Mid septal	-19.79 ± 3.90*	-1.10 ± 0.23	1.49 ± 0.37^	1.00 ± 0.35
Basal septal	-19.05 ± 3.60*	-1.14 ± 0.21	1.57 ± 0.45^	1.06 ± 0.35
RV-Freewall	-28.50 ± 6.34Ÿ	-1.97 ± 0.56Ÿ	2.16 ± 0.69^	1.45 ± 0.53u
Male				
Apical lateral	-24.66 ± 5.94	-1.55 ± 0.41	1.76 ± 0.56	1.33 ± 0.50
Mid lateral	-27.82 ± 5.35	-1.70 ± 0.45	1.61 ± 0.46	1.27 ± 0.37
Basal lateral	-27.24 ± 7.33	-1.93 ± 0.63	2.02 ± 0.76	1.44 ± 0.52
Apical septal	-18.07 ± 5.41	-1.32 ± 0.44	1.64 ± 0.55	1.11 ± 0.47
Mid septal	-17.83 ± 4.08	-1.04 ± 0.23	1.29 ± 0.34	1.03 ± 0.30
Basal septal	-17.80 ± 3.34	-1.13 ± 0.22	1.36 ± 0.39	1.10 ± 0.34
RV-Freewall	-26.50 ± 6.32	-1.80 ± 0.59	1.80 ± 0.63	1.35 ± 0.472

*Vs. male apical septal p<0.05 ^Vs. Male results p<0.001

†Vs. male apical lateral <0.001 Ÿvs. Male RV-Freewall p<0.01

**Vs. male apical lateral <0.01 uvs. Male RV-Freewall p <0.05

3.13. 3D Volume Results

3D acquisition was conducted in each of the 266 healthy volunteers recruited to the study. Volume measurement, using the TomTec system, was possible in 191 (72%) people. The group demographics were similar to those previously reported. Mean group age was 40±11 years, height 1.66±0.9 m, mass 69.51±12.9 kg, with a BSA and BMI of 1.78±0.20 m^2 and 24.97±3.6 kg/m respectively.

Table 3.29 shows the group results organised by gender. Male results were larger than females resulting in highly significant difference between male and female 3D volumes both at baseline and when ratio scaled to BSA. Females demonstrated a higher ejection fraction.

Table 3.29 3D volume results organised by gender

	Total Mean±SD	Female (n=86) Mean±SD	Male (n=105) Mean±SD	p
3D ESV (ml)	57 ± 18.20	48 ± 16.08	65 ± 16.35	<0.0001
3D EDV (ml)	123 ± 35.36	106 ± 31.42	136 ± 32.38	<0.0001
3D EF (%)	53 ± 5.87	54 ± 5.81	52 ± 5.80	<0.05
3D SV (ml/min)	65 ± 20.23	57 ± 17.84	72 ± 19.83	<0.0001
3D ESV/BSA (ml/m2)	32 ± 8.85	29 ± 8.89	34 ± 8.03	<0.0001
3D EDV/BSA (ml/m2)	68 ± 17.11	63 ± 17.47	72 ± 15.75	<0.0001
3D SV/BSA (ml/min/m2)	36 ± 10.14	34 ± 10.16	38 ± 9.87	<0.05

3.13.1. Relationship with age and gender

Results of the bivariate correlations between RV volumes and anthropometric data are shown in Table 3.30. Age showed a significant, decreasing trend across RV EDV, ESV and SV. EF also demonstrated a negative but non-significant correlation. Height

body mass and BSA showed similar correlations across all RV volumes and RV SV, with height alone displaying a non-significant relationship with RV EF. BMI demonstrated minimal and non-significant relationships with both RV EDV and ESV and consequently RV SV as well.

Like both body mass and BSA, BMI displayed a significant inverse relationship with EF ($p < 0.05$). BMI consistently showed the weakest correlation. The lack of association to body size measurements resulted in BMI being excluded from the multivariable analysis.

Table 3.30 Bivariate 3D correlations between RV data and anthropometric measurements

	RV EDV	RV ESV	RV SV	RV EF
Age	-0.46*	-0.40*	-0.45*	-0.09
Height	0.59*	0.56*	0.53*	-0.08
Body mass	0.48*	0.49*	0.40*	-0.17♯
BMI	0.13	0.17	0.07	-0.15♯
BSA	0.53*	0.53*	0.45*	-0.16♯

EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; RV, right ventricular; and SV, stroke volume. *correlation is significant at the 0.01 level; ♯correlation is significant at 0.05 level.

The correlation between the RV volumes and EF shown in Table 3.30 prompted the need to scale this data. The common clinical practice is to do this using a ratio-indexing approach ($s=y/x$) using each of the body size parameters (Oxborough et al., 2012b).

Ratiometric scaling of RV parameters to height, body mass, BSA and BMI are shown in Table 3.31. RV measurements maintained a degree of significant residual correlations with both height and body mass, even after indexing to BSA or BMI. Of note, height as the scaling variable, demonstrated a positive correlation and a resultant lack of size independence across all volume measurements, and associated 3D EF ($r>0.3$ $p<0.001$).

3D RV volumes and EF scaled to body mass resulted in a weaker (r=0.16) but significant inverse relationship for RV EDV. RV ESV however displayed a lack of correlation. 3D RV EF displayed a significant inverse correlation with body mass.

3D volumes scaled to BSA did result in some size independent measurements but these did not remain consistent across all 3D measurements and therefore it was appropriate to scale these 3D measurements allometrically. To further confirm this assumption, when the data was tested in the manner described previously, Tanners 'Special circumstance' was also not satisfied.

Further analysis was undertaken using BSA and height as the primary scaling factors. This was based on the historical use of height along with the strong association for linear relationships, and the now commonplace use of BSA as the scaling variable of choice across different studies and normal reference ranges (Oxborough et al., 2012b, Lang et al., 2005).

Table 3.31 Ratiometric scaling of 3D RV measurements displaying significant correlation post-scaling for many of the scaled combinations.

scaled variable	Height	Mass	BSA	BMI
RV EDV				
RV EDVraw	0.59**	0.48**	0.53**	0.13
RV EDVheight	0.42**	0.39**	0.42**	0.14
RV EDVmass	0.18*	-0.16*	-0.1	-0.38**
RV EDVBSA	0.33**	0.12	0.17*	-0.13
RV EDABMI	0.54**	0.11	0.22**	-0.31**
RV ESV				
RV ESVraw	0.57**	0.50**	0.54**	0.16*
RV ESVheight	0.42**	0.41**	0.43**	0.18*
RV ESVmass	0.20**	-0.09	-0.03	-0.30**
RV ESVBSA	0.33**	0.17*	0.21**	-0.06
RV ESABMI	0.53**	0.15*	0.25**	-0.24**
RV EF				
RV EFraw	-0.08	-0.17*	-0.15*	-0.16*
RV EFheight	-0.54**	-0.44**	-0.50**	-0.13
RV EFmass	-0.57**	-0.85**	-0.85**	-0.66**
RV EFBSA	-0.58**	-0.75**	-0.77**	-0.51**
RV EFBMI	-0.07	-0.65**	-0.56**	-0.80**
RV SV				
RV SVraw	0.53**	0.40**	0.45**	0.07
RV SVheight	0.38**	0.31**	0.34**	0.07
RV SVmass	0.15*	-0.20**	-0.13	-0.39**
RV SVBSA	0.28**	0.05	0.10	-0.16*
RV SVBMI	0.48**	0.05	0.16*	-0.36**

EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; SV, stroke volume. *correlation is significant at ≤0.01, ** correlation is significant at ≤ 0.0001.

Subsequent allometric analysis of the relationships between 3D RV volumes and BSA, revealed diverse b exponents and confidence intervals, ESV, b = 1.51 (95% CI = 1.16 to 1.86); EDV, b = 1.36 (95% CI = 1.04 to 1.68); SV, b = 1.23 (95% CI = 0.88 to 1.59). The process was repeated for height. ESV, b = 3.10 (95% CI = 2.4 to 3.7); EDV, b = 2.9 (95% CI = 2.3 to 3.5); SV, b = 2.7, (95% CI = 2.13 to 3.45).

Further calculation of b and c exponents in an attempt to ascertain the association between ethnicity and RV volumes and BSA/height, yielded very small c values. These ranged from 0.03 to 0.05 for BSA ESV and height SV respectively. Calculated b values yielded no significant differences from previously calculated b values (p ns).

The use of this data in $y=a.x^b$ scaling of RV volumes to BSA did subsequently produce size independent indices for all volume measurements, (ESV r= 0.001 p =0.988; EDV, r=0.007, p =0.92; SV, r=0.01, p =0.88). Similar results were found with height (ESV, r=-0.01, p =0.99; EDV, r=0.015, p =0.84; SV=0.03, p =0.61). Figures 3.5 to 3.10 are scatter plots highlighting both the ratio and allometric relationships for EDV, ESV and SV for both BSA and height.

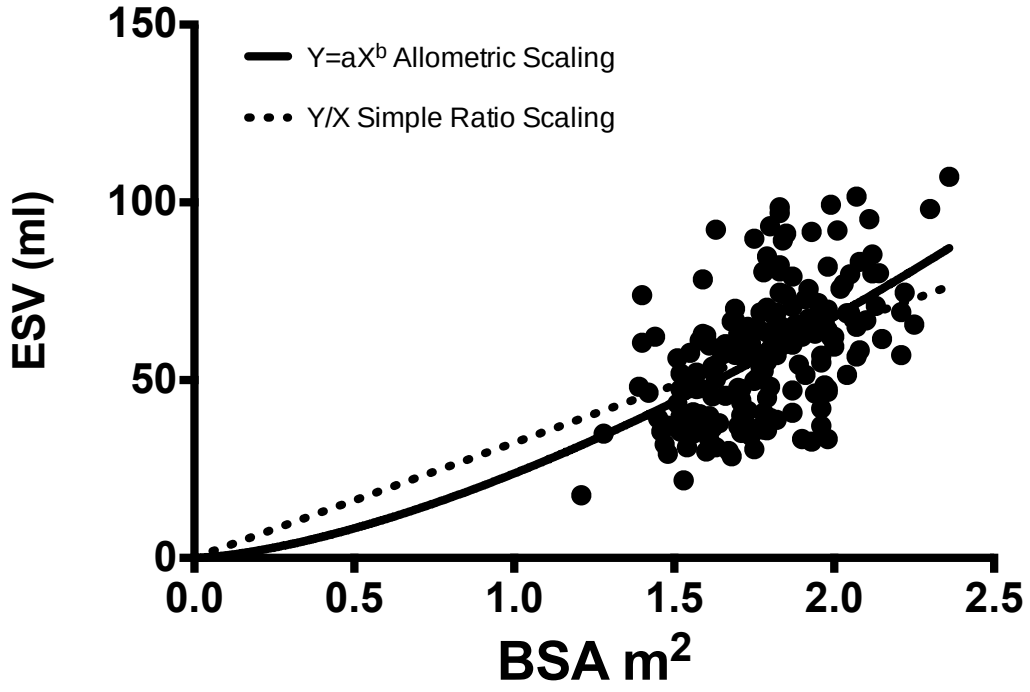

Figure 3.5 Scatter plot showing both ratiometric and allometric regression plots for scaled BSA, 3D acquired ESV.

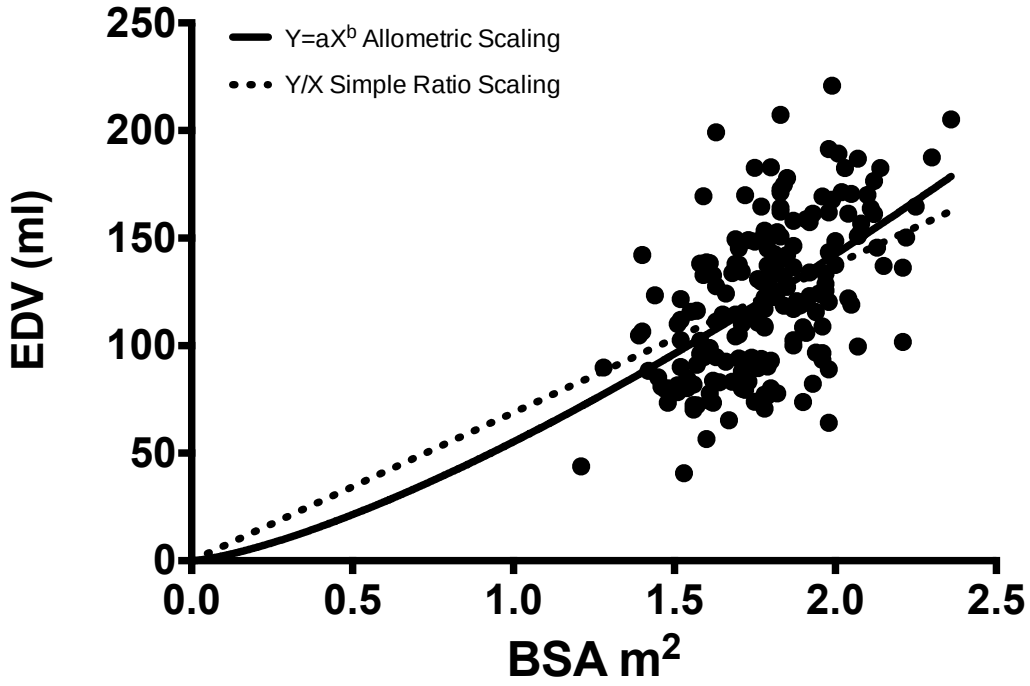

Figure 3.6 Scatter plot showing both ratiometric and allometric regression plots for scaled BSA, 3D acquired EDV

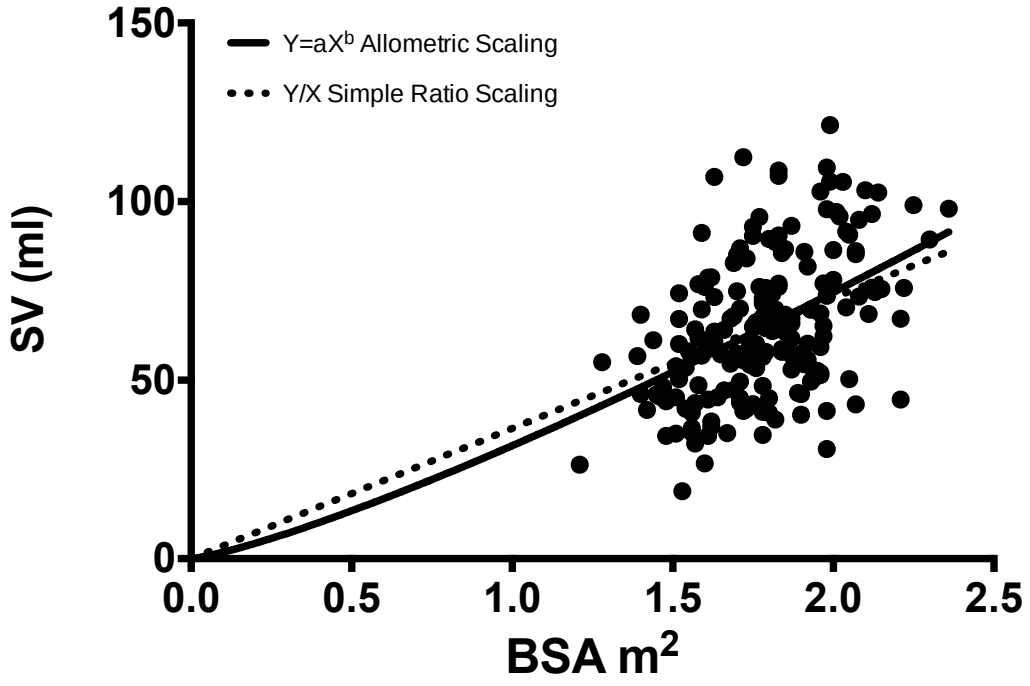

Figure 3.7 Scatter plot showing both ratiometric and allometric regression plots for scaled BSA, 3D acquired SV

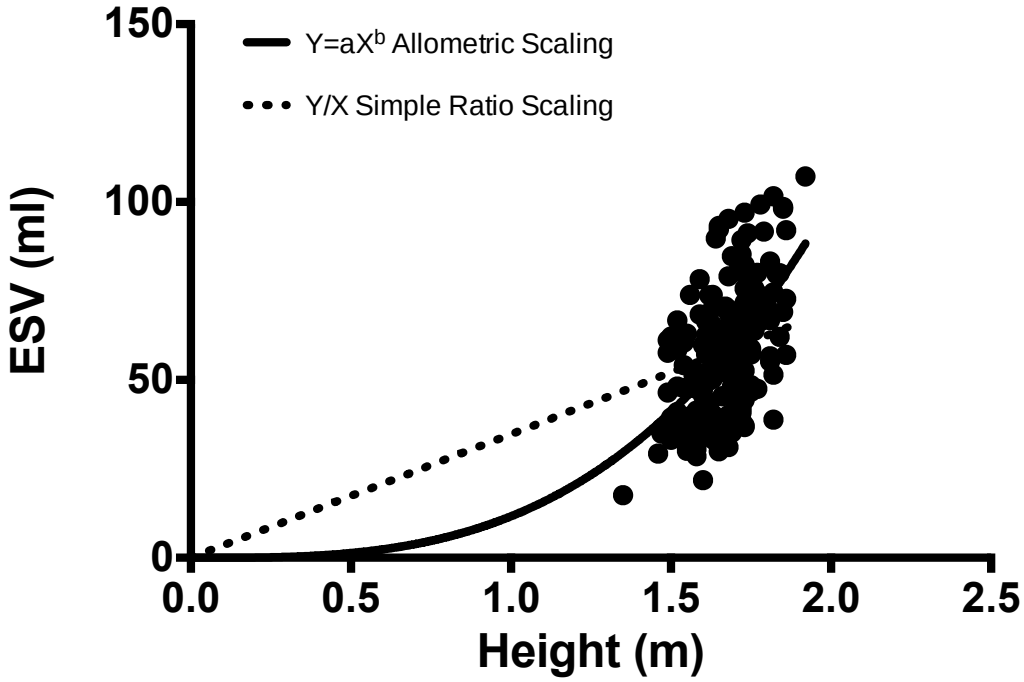

Figure 3.8 Scatter plot showing both ratiometric and allometric regression plots for scaled height, 3D acquired ESV.

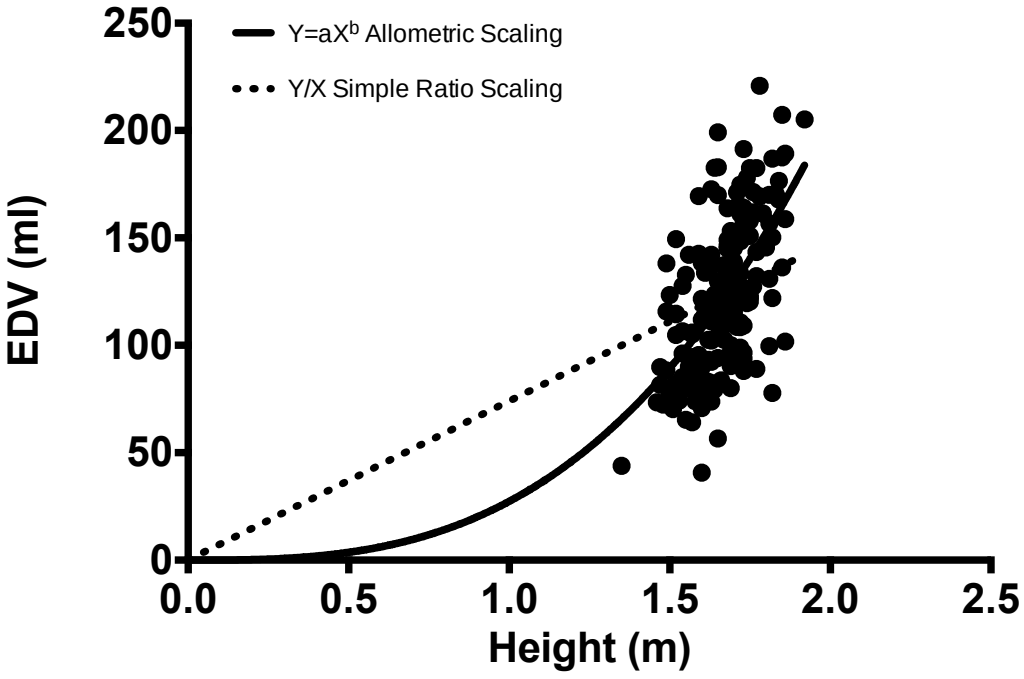

Figure 3.9 Scatter plot showing both ratiometric and allometric regression plots for scaled height, 3D acquired EDV.

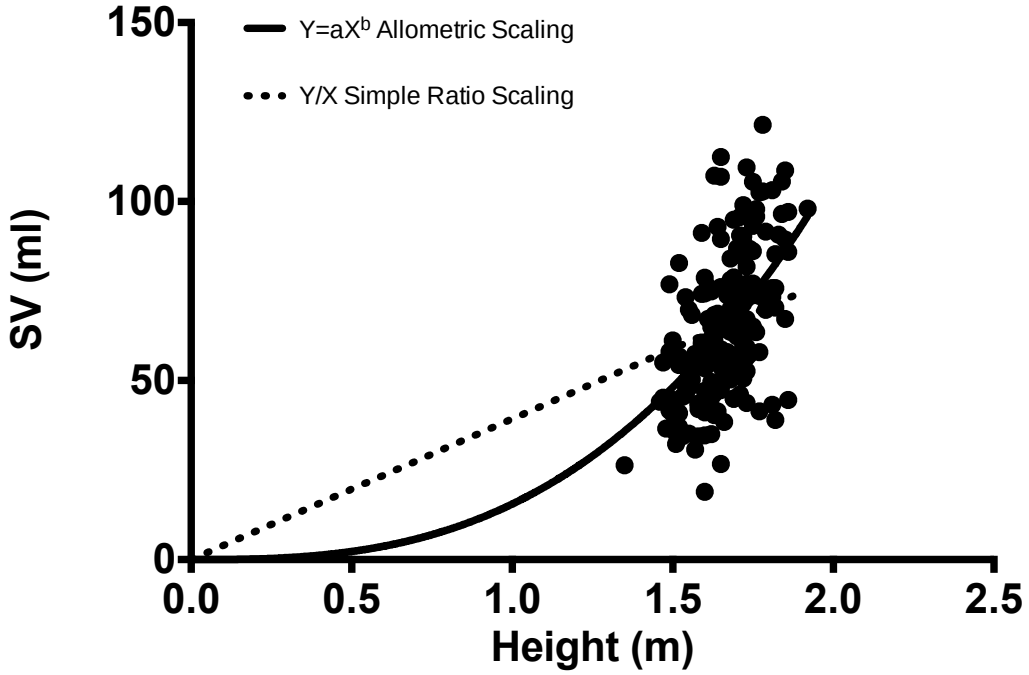

Figure 3.10 Scatter plot showing both ratiometric and allometric regression plots for scaled height, 3D acquired SV.

Subsequent regression analysis was conducted using both absolute RV volume data and measurements scaled using allometric processes to both height and BSA. The results of the 3D volume, multiple regression analysis are shown in Tables 3.32–3.35. These encompass the raw data, and allometric-scaled results scaled to height and BSA.

Table 3.32 Results of a one way ANOVA organised by gender for RV 3D volumes.

	3D ESV (ml)									3D EDV (ml)									3D EF (%)				3D SV (ml)								
	n	Female				n	Male				Female				Male					Female				Female				Male				
Afro-Caribbean	21	61	±	17		26	70	±	15		136	±	33		154	±	26			54	±	6	54	±	7	73	±	19		84	±	18	
European	16	50	±	11		20	78	±	16		107	±	19	°	160	±	31			54	±	7	51	±	5	57	±	14	*	82	±	18	
Chinese	19	42	±	18	*	22	58	±	10	Ÿ	89	±	30	*	120	±	25	†n		54	±	7	51	±	5	47	±	15	*	61	±	18	Ÿ‡
Indian	17	43	±	11	♯	17	57	±	16		97	±	24	*	119	±	28	†n		54	±	5	52	±	6	55	±	14	*	64	±	16	± ‡
Malays	13	42	±	9	*	20	60	±	15	^n	91	±	19	*	122	±	27	†n		54	±	3	51	±	6	49	±	10	°	62	±	16	^n

* p value <0.001compared to Afro-Caribbean females, °p value <0.05 compared to Afro-Caribbean females; ♯p value<0.01 compared to Afro-Caribbean females; Ÿp <0.01 compared to European males; ^ p <0.0001 compared to European males; np <0.001 compared to Afro-Caribbean males; †p <0.001 compared to European males; ± p <0.05 compared to European males; ‡ p 0.01 compared to Afro-Caribbean males

Table 3.33 Regression tables for simple 3D RV data

	ESV ($R^2$47%)				EDV ($R^2$52.9%)				SV ($R^2$46.5%)				EF ($R^2$11%)			
	b	95% CI		p	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference															
Male	11.04	5.54	16.55	<0.001	18.87	8.78	28.96	<0.001	8.05	1.95	14.16	0.01	-1.57	-3.82	0.67	ns
Height	23.83	-9.73	57.40	ns	65.19	3.62	126.75	0.04	43.08	5.82	80.35	0.02	3.91	-9.78	17.60	ns
Mass	0.26	0.05	0.47	0.02	0.32	-0.07	0.71	ns	0.04	-0.19	0.28	ns	-0.10	-0.19	-0.02	0.02
< 29 yrs	Reference															
<39 yrs	-5.94	-12.43	0.56	ns	-8.49	-20.40	3.42	ns	-2.33	-9.54	4.88	ns	1.12	-1.53	3.77	ns
<49 yrs	-7.99	-13.88	-2.11	0.01	-19.31	-30.11	-8.51	<0.001	-11.00	-17.54	-4.47	<0.001	-1.19	-3.60	1.21	ns
<59 yrs	-5.21	-12.30	1.88	ns	-14.39	-27.39	-1.39	0.03	-10.10	-17.97	-2.24	0.01	-2.42	-5.31	0.47	ns
60+ yrs	-17.24	-26.93	-7.55	<0.001	-33.95	-51.73	-16.18	<0.001	-16.63	-27.39	-5.87	<0.001	-0.06	-4.01	3.90	ns
European	Reference															
Indian	-6.23	-13.58	1.13	0.10	-12.98	-26.47	0.52	ns	-6.62	-14.78	1.55	ns	-0.02	-3.02	2.98	ns
Chinese	-7.05	-14.27	0.16	ns	-9.31	-22.54	3.92	ns	-1.11	-9.12	6.90	ns	1.65	-1.29	4.59	ns
Malay	-5.91	-12.92	1.10	ns	-11.67	-24.53	1.19	ns	-6.08	-13.86	1.71	ns	0.30	-2.56	3.16	ns
AfroCar	3.02	-3.36	9.40	ns	13.36	1.65	25.06	0.03	10.12	3.04	17.21	0.01	2.24	-0.37	4.84	ns
	p <0.001				p <0.001				p <0.001				p <0.001			

European, Female and <29 years were used as reference groups.

Table 3.34 Regression tables for 3D volumes allometrically scaled to BSA

	ESV/BSA1.51 ($R^2$17%)				EDV/BSA1.36 ($R^2$23%)				SV/BSA1.23 ($R^2$23.5%)			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference											
Male	2.94	1.1	4.8	<0.001	5.05	1.32	8.77	0.01	2.13	-0.24	4.50	ns
< 29 yrs	Reference											
<39 yrs	-3.08	-5.9	-0.2	0.03	-6.03	-11.83	-0.23	0.04	-2.77	-6.46	0.91	ns
<49 yrs	-3.45	-6.0	-0.9	0.01	-9.85	-15.01	-4.69	<0.001	-6.44	-9.72	-3.16	<0.001
<59 yrs	-2.40	-5.5	0.7	ns	-7.92	-14.23	-1.61	0.01	-6.12	-10.13	-2.11	<0.001
60+ yrs	-7.17	-11.4	-2.9	<0.001	-16.02	-24.70	-7.34	<0.001	-8.81	-14.33	-3.30	<0.001
European	Reference											
Indian	-1.32	-4.4	1.8	ns	-3.29	-9.60	3.01	ns	-1.94	-5.94	2.07	ns
Chinese	-1.83	-5.0	1.3	ns	-1.68	-8.04	4.68	ns	0.88	-3.16	4.92	ns
Malay	-1.50	-4.5	1.5	ns	-2.76	-8.95	3.43	ns	-1.43	-5.36	2.51	ns
AfroCar	1.00	-1.8	3.8	ns	5.22	-0.54	10.99	ns	4.34	0.68	8.00	0.02
	p <0.001				p <0.001				p <0.001			

European, Female and <29 years were used as reference groups.

Table 3.35 Regression tables for 3D RV volumes allometrically scaled to height

	ESV/HT3.10 ($R^2$12%)				EDV/HT2.9 ($R^2$19%)				SV/HT2.7 ($R^2$22%)			
	b	95% CI		p	b	95% CI		p	b	95% CI		p
Female	Reference											
Male	0.94	-0.08	1.96	ns	1.59	-0.48	3.65	ns	0.74	-0.59	2.06	ns
Mass	0.00	-0.04	0.04	ns	-0.03	-0.11	0.05	ns	-0.04	-0.09	0.02	ns
< 29 yrs	Reference											
<39 yrs	-0.82	-2.23	0.59	ns	-1.34	-4.20	1.52	ns	-0.42	-2.25	1.41	ns
<49 yrs	-0.71	-1.96	0.54	ns	-2.67	-5.21	-0.13	0.04	-2.04	-3.66	-0.41	0.01
<59 yrs	-0.53	-2.06	1.00	ns	-2.24	-5.33	0.86	ns	-2.10	-4.08	-0.12	0.04
60+ yrs	-2.74	-4.84	-0.64	0.01	-6.07	-10.32	-1.81	0.01	-3.38	-6.10	-0.65	0.02
European	Reference											
Indian	-0.26	-1.82	1.30	ns	-0.98	-4.14	2.19	ns	-0.77	-2.79	1.26	ns
Chinese	-0.46	-2.02	1.10	ns	-0.17	-3.33	2.99	ns	0.63	-1.39	2.65	ns
Malay	-0.79	-2.32	0.74	ns	-1.80	-4.90	1.30	ns	-1.14	-3.12	0.85	ns
AfroCar	1.09	-0.31	2.49	ns	3.95	1.11	6.79	0.01	2.97	1.15	4.79	<0.001
	p <0.001				p <0.001				p <0.001			

European, Female and <29 years were used as reference groups.

3.14. 3D Volumes and Ethnicity

Simple ANOVA analysis displayed significant differences between ethnic groups and 3D RV ESV EDV and SV ($p < 0.001$). There was no significant difference in 3D EF between groups. Both Afro-Caribbean and European groups displayed larger ESV ($p < 0.001$), EDV ($p < 0.001$) and SV ($p < 0.001$) compared to the remaining ethnic groups.

Table 3.32 displays the 3D results of a simple ANOVA organised by gender and ethnicity, highlighting the significant relationships across the gender/ethnic groups. Of note is the fact that Afro-Caribbean females displayed significantly increased EDV, ESV and SV compared to Indian, Chinese and Malay participants. For males, both European and Afro-Caribbean results were significantly larger in various permutations than the remaining ethnic groups.

A highly significant gender based bias was identified within the absolute data set, with males displaying larger ESV, EDV and SV ($P < 0.01$). This was replicated in both EDV and ESV within the regressed BSA(exp) results. Results scaled to height(exp) displayed gender independence.

Regression analysis conducted on the absolute RV data (Table 3.33) demonstrated a decreasing trend in RV volumes with increased age (per decade). In volunteers 50 years old and over ESV demonstrated a significant decrease, compared to the reference group. EDV displayed a similar decreasing trend, significant in the $\leq$49 year and 60+ groups ($p < 0.01$). In concordance with this, SV also decreased with increasing age over 49 years. Similar results were still evident after allometrically scaling results to BSA(exp) but were not so evident when scaled to height(exp) (Table 3.34 & 3.35 respectively).

Regression results displayed a limited statistical impact on ethnic variation across the group. With the exception of the Afro-Caribbean group, all ethnicities displayed

reduced EDV and ESV and SV compared to the reference European group shown in Table 3.33 however these remained non-significant.

The Afro-Caribbean group, after controlling for several other cofounders, displayed a larger EDV and SV (EDV b =13.36ml, p <0.05 & SV b =10.12ml, p =0.01) than the reference group (p <0.05). Regression conducted using the BSA(exp) allometrically scaled data, displayed a significant ethnic difference between the Afro-Caribbean and European groups SV (b =4.34ml, p <0.05), whereas these two groups displayed significant differences for EDV (b =3.95ml, p <0.01) and SV (b =2.97ml, p <0.001), scaled to height(exp).

URV for RV volumes derived from the raw study data and allometric processes are displayed in Table 3.36 with a 95% reference range for RVEF of 41-63% for males and 43-65% for females.

Table 3.36 95% URV for gender stratified RV volumes. Results displayed are both unadjusted raw data URV and allometric scaled URV with calculated beta values displayed in bold.

	Male	Female
RV ESV	97 ml	80 ml
RV ESV $^{(EXP)}$	37 ml/$m^{2\ (\mathbf{1.51})}$	36 ml/$m^{2\ (\mathbf{1.51})}$
RV EDV	200 ml	167 ml
RV EDV $^{(EXP)}$	83 ml/$m^{2\ (\mathbf{1.36})}$	81 ml/$m^{2\ (\mathbf{1.36})}$
RV SV	110 ml	92 ml
RV SV $^{(EXP)}$	49ml/$m^{2\ (\mathbf{1.23})}$	48ml/$m^{2\ (\mathbf{1.23})}$

3.15. 3D volume repeatability results

Table 3.37 Repeatability results across all three test scenarios.

	Intraobserver			Interobserver			Retest		
	COV (%)	ICC	95% LOA	COV (%)	ICC	95% LOA	COV (%)	ICC	95% LOA
ESV (ml)	8	0.96	-12.5 to 9.6	13	0.92	-15.5 to 18.2	12	0.90	-17.8 to 16.49
EDV (ml)	6	0.96	-18.7 to 18.5	12	0.89	-28 to 38	9	0.91	-25.26 to 30.11
EF (%)	8	0.82	-7.3 to 9.0	11	0.45	-10 to 12	9	0.73	-8.36 to 10.97
SV (ml)	12	0.89	-16.9 to 19.5	21	0.66	-25 to 32	13	0.85	-17.61 to 23.79

Table 3.37 shows the combined repeat analysis conducted on the RV 3D volume data. Intraobserver 3D results showed no systematic bias. All ICC results were scored as very good with EDV displaying the highest score of 0.96. COV scores ranged from 6% to 12%. Intraobserver EDV displayed the widest LOA at +18.7ml. Interobserver results displayed an increase in COV measurements and no systematic bias. ICC scores indicated very good agreement for both ESV and EDV with good scores for both SV and EF respectively.

The LOA were wider compared to the intraobserver results with EDV displaying the widest agreement. Retest or acquisition testing results, displayed improved ICC scores compared to interobserver results for EDV, EF and SV. COV scores ranged from 9% to 13% whilst also displaying acceptable LOA.

4. Discussion

This observational study aimed to investigate RV dimensions, function and volume using a range of quantitative tools. The study sample comprised of healthy volunteers from a range of ethnic backgrounds. These volunteers underwent a standard transthoracic exam that featured assessment of RV dimensions using 2D calliper, 3D volume and post-processed myocardial speckle tracking.

In addition to standard linear dimensions, this study investigated the importance of indexing results to BSA and the determination of normal values for a range of ethnic groups split by gender. The results highlight two main considerations when assessing the RV. Firstly, whilst there are no significant differences in functional 2D RV echocardiographic parameters, there is extensive disparity in the simple linear RV dimensions between males and females across a range of measurements.

The second consideration surrounds the influence that techniques such as ratio scaling to BSA and ethnicity can have on right heart dimensions. In addition to assessing the normal data within this group of healthy volunteers, we have reported the reproducibility statistics for each of the three measurement criteria in order to provide an accurate assessment of these diagnostic tools in a clinical setting.

The total number of subjects recruited during the course of the investigation was 302. Of these, 15 volunteers were excluded from the study due to pathology - either found at interview or with screening echocardiography. Volunteers were rejected due to a history of using hypertension medication, LVH on screening or greater than mild valvular regurgitation. Those requiring medical supervision were placed under the care of a local cardiologist or general practitioner. One case of Wolf-Parkinson-White was found on a resting ECG and was subsequently referred direct for cardiology follow-up.

32 further volunteers were removed post-recruitment, due to poor image quality across all echocardiographic windows, rendering their studies redundant.

4.1. Image quality

The difficulty in acquiring suitable RV images with correct wall delineation throughout the cardiac cycle, has been well documented (Haddad et al., 2008, Mertens and Friedberg, 2010), and is a potential limitation to the use of echocardiography as an assessment tool. The use of anatomical markers at the annulus was introduced in an effort to standardise repeat measurements. When assessed this produced acceptable levels of repeatability and is discussed in section 4.5.

Table 3.3 shows the measurement acquisition rate across both ethnic groups and RV calliper measurements. As a group, the Malay population had the lowest rate of successful image acquisition within the apical window. During scanning, acquisition reductions in RV lateral wall delineation were believed to be due to smaller intercostal spacings limiting the echocardiographic windows through which adequate imaging could be achieved. This was a simple observation of the sonographers involved, despite adjusting the position of the volunteers and imaging throughout normal respiration to ascertain the optimal window.

4.2. 2D Linear Measurements

This study is believed to be the first echocardiographic study since the work of Foale et al in 1986 (shown in figure 4.0) (Foale et al., 1986) to undertake dedicated measurements of the RV in order to obtain normal values prospectively, as a primary aim, and not as part of a control group. The chronological basis for the common references found in RV linear dimension studies often involves the work of Foale and colleagues. Adaption of these measurements into the 2005 ASE/EAE guidelines provided a simple reference for RV assessment featuring RVD1-3 in the apical window and RVOT1-2 in the parasternal window. Despite this, the location of these measurements appears to lack the anatomical clarity often provided with LV assessment.

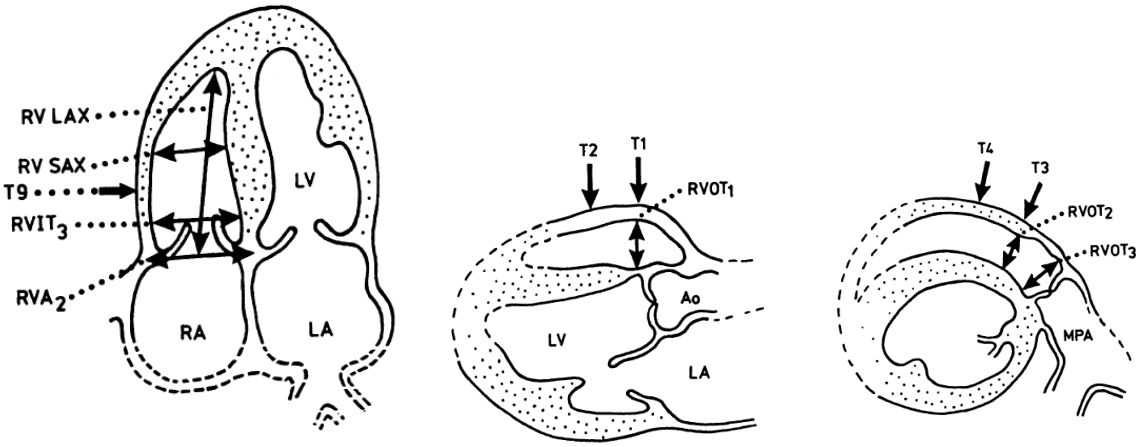

Figure 4.0 Original diagrams showing the location of each of Foale's RV measurements (Foale et al., 1986).

To ensure that we provided both robust and reproducible measurements Foale's et al.'s original paper was used as the reference for the location of the RV measurements, advocated by both 2005 and 2010 guidelines (Lang et al., 2005) (Rudski et al., 2010).

Within the literature surrounding RV assessment there also appears to be either a lack of consistency or a lack of understanding regarding the location of these measurements. In particular, the location of RVD-2 has been questioned, (Valsangiacomo Buechel and Mertens, 2012) with Foale suggesting that the measurement be made within the body of the RV at the widest point, whilst Lang et al refer to the location of RVD-2 as level with the LV papillary muscle (Lang et al., 2005).

The comparison of results from between studies shown in Table 4.0 identify a better level of agreement between the URV results of this thesis and the works of Foale and colleagues (1986) compared to that of Lang (2005), with RVD-2 measurements from both former studies exceeding that of the latter. Despite the acknowledgement that this measurement be made at the level of the LV papillary muscle, the use of Foale's mid cavity proved to be the favoured technique, better representing the anatomical differences between the two ventricles.

Table 4.0 Comparison of several RV study, 95% Upper reference values (URV)

	Linear							Ratiometric					Allometric
	Lang (2005)	Foale (1986)	Rudski (2010)	Willis (2014)				Foale (1986)	Willis (2014)				Willis (2014)
(mm)				Male	Female	Group	(mm/m2)		Male	Female	Group	(mm/m2(exp))	
RVOT -1	≤29	≤32	≤35	≤38	≤35	≤37	RVOT -1	≤20	≤20	≤21	≤21	RVOT-1 (mm/m2(0.55))	≤27
RVOT - 2	≤23	≤26	≤27	≤28	≤26	≤30	RVOT - 2	≤14	≤15	≤16	≤15	RVOT-2 (mm/m2(0.55))	≤19
RVOT-3	-	≤30	≤33	≤33	≤31	≤33	RVOT-3	≤17	≤17	≤19	≤18	RVOT-3 (mm/m2(0.63))	≤22
RV-WT	-	≤5	≤5	≤6	≤6	≤6	RV-WT	≤3	≤3	≤3	≤3	RV-WT (mm/m2(0.36))	≤4
RVD-1	≤28	≤30	≤42	≤43	≤40	≤42	RVD-1	≤18	≤22	≤24	≤23	RVD-1 (mm/m2(0.67))	≤27
RVD-2	≤33	≤37	≤35	≤39	≤37	≤39	RVD-2	≤22	≤22	≤23	≤22	RVD-2 (mm/m2(0.33))	≤29
RVD-3	≤79	≤89	≤86	≤90	≤83	≤88	RVD-3	≤54	≤49	≤52	≤50	RVD-3 (mm/m2(0.38))	≤70
RVD-AN	-	≤31	-	≤36	≤33	≤35	RVD-AN	≤18	≤19	≤19	≤19	RVD-AN (mm/m2(0.84))	≤21
RVESA	≤16	-	≤14	≤15	≤12	≤14	RVESA (cm/m2)	-	<8	≤7	≤7	RVESA (cm/m2(1.61))	≤5
RVEDA	≤28	-	≤25	≤27	≤22	≤26	RVEDA (cm/m2)	-	<14	≤13	≤13	RVEDA (cm/m2(1.37))	≤11

The trabeculated surface of the RV presents false borders and crypts that need to be identified and excluded within such measurements as RVD-2 and RVD-3.

The suggestion that mere increases in RV dimensions compared to Langs (2005) study alone equal a more accurate representation of the chamber size is a limited approach to the interpretation of the data. The acknowledgement of the similarities between both Foale et al (1986) as the original data source and that of Rudski et al (2010) with whom improved imaging techniques will have allowed for better identification of false epicardial borders and trabeculations within the apex, support these increased, multifaceted URVs.

In further support of this approach work conducted by Triulzi et al (1984) also identified in their study of 72 volunteers the mid chamber approach (RVD-2) to RV assessment resulted in narrower dimensions compared to the basal measurements (RVD-1) prompting the need, as clearly identified within this thesis, to state categorically the approach taken with RV assessment.

This important early paper was one of the first to systematically assess the all four chambers using a standardised approach and detailed methodology. In addition the importance of both gender, age and body were also investigated using linear regression modelling. Interestingly however, there was a lack of gender difference after correcting for these associated variables, with no significant difference between males and females. This may have resulted from either a single or combined effect of smaller sample size (*n* 72) or a more homogenous group within assessment. Despite this contrast in findings between studies, this thesis and that of both Foale et al (1986) and Rudski et al (2010) support the use of a mid cavity measurements for the body of the RV at RVD-2 level.

In order to replicate Foale's et al.'s methodology we utilised the measurement of RVA2, (renamed RVD-AN in reference to the RV annulus) a measurement made at TV annular level. Changes in TV annular dimensions are often associated with changes in both RV size and function, particularly in patients in which tricuspid regurgitation is more than mild (Lancellotti et al., 2010). Consequently, annular measurements provide guidance for valve repair or replacement (Dreyfus et al., 2005),

with multiple measurements often made via consecutive echocardiographic studies, prior to surgical intervention. This may be linked to primary left sided heart disease with the RV changing in size and shape as a consequence of the failing left heart. With a potential lack of patient symptoms, the assessment of annular dimensions may provide some guidance on the extent of RV involvement which may result in additional surgical intervention.

This left and right heart interaction, described earlier in physiological terms as ventricular interdependence, provides a good example where understanding factors that may cause the right heart to change in both size and or function can both guide and aid decision making in the use of standard tools such as calliper measurements.

In addition to the functional measures already described, qualitative techniques such as the visual estimation of RV size and function to that of the LV are essential in helping to build the complete chamber assessment. The morphology of the RV presents with a more tortuous outline than the LV given the trabeculations and false tendons that exist. Experience in excluding these within both calliper, area and volume measurements are essential for the most basic of scans whilst obtaining the correct orientation.

Despite the similar mid cavity measurements shown in RVD-2, the focused RV view advocated by Rudski et al (2010) may have resulted in a more accurate representation of the inflow portion of the RV. This thesis then presenting with wider basal dimensions resulting in the larger RVD-1 measurements (shown in Table 4.0) between both Foale et al (1986) and Lang et al (2005) but very similar results compared to Rudski et al (2010). The impact that variations in these particular dimensions could have on surgical interventions should not be underestimated further highlighting the importance of identifying the correct plane in which measurements should be made.

The introduction of this annular measurement also aimed to standardise the calculation of RVD-3 by providing anatomical markers at both the annular and apical level. In addition, it was felt that this would help remove some of the apparent misrepresentation surrounding the placement of RVD-1, found at the widest point in

the basal third section of the cavity. This demonstrated acceptable intra- and interobserver ICC scores, shown in Table 3.23, however, retest analysis demonstrated poor reliability for RVD-3 measurements with ICC scores <0.2 and it was also associated with wider LOA and increased COV compared to the repeat image testing.

These results suggest that the acquisition of the RV images remains an important factor in the determination of RV length dimensions and may be subject to foreshortening, often to subtle to be detected. This issue appears, however, to be isolated to the apical measurement RVD-3 with the remaining measurements displaying acceptable levels of reliability and measurement variability.

The apex of the RV is often determined to lie below that of the 'apex forming' LV (Rudski et al., 2010). The acquisition protocol used for each study required the use of an RV focused view. Optimising the orientation of the RV between scans may have resulted in a different presentation of the true apex, resulting in either underestimation or overestimation of the true base to apex length.

This apical image and the resulting measurements have been described as frequently foreshortened on echocardiography, resulting in limited assessment of the true cardiac apex (Lang et al., 2005). The basal and mid measurements may be similarly reduced due to transducer location when compared to cMRI (Lai et al., 2008). The subsequent basal and mid measurements were found to be reduced when compared to intraobserver and interobserver results however the divergence between studies may be regarded as relative to the acquisition error.

A number of technical challenges were found in the acquisition of these key measurements. These may be limited to this study only, but discussion of these challenges is important in order to understand where potential sources of measurement variation may be introduced. A number of dimensions from studies of the healthy volunteer subject group measured in excess of the representative 2005 guidelines for RV size. Although these recommendations have been surpassed during the time of this study by new ASE guidelines, as discussed in this thesis, this further highlights the need for quantitative data to be collected.

Currently the use of 2D calliper measurements in quantifying RV size remains under-reported (Kjaergaard et al., 2006b). In addition to this suggestion of under-reporting, within the current study the number of measurements acquired that fell outside of the normal range based on the 2005 guidelines was, up to 69% seen in RVD-1.

When compared to the 2010 ASE guidelines, the number of dilated normal studies reduced from 69% to 3% (specifically for RVD-1). As a result, it was recognised within the 2010 guidelines that it was not appropriate to use the reference range data derived from Foale et al (Foale et al., 1986) original study as presented in Lang's 2005 paper (Rudski et al., 2010, Lang et al., 2005).

Table 4.0 illustrates the increase in both basal and longitudinal upper reference linear RV measurements from each of the aforementioned RV studies. Reference values suggesting increased RV length and width (mainly RVD-3 and RVD-1) found in both RV guidelines (Rudski et al., 2010) and data derived from this study may result from several variables. In addition, this table provides the first complete assessment of RV 2D calliper measurements available for both gender where appropriate, with indexed measurements and allometric upper reference values (URV).

Quoting measurements indexed to BSA remains common practice in some centres, despite the associated measure falling within standard reference ranges. It is anticipated that the allometric data presented in Table 4.0 will be used to substantiate measurements that fall outside of current normal values, for example in tall athletic patients or in those with whom the RV appears disproportionately large but may not exceed normal values.

Further evidence in support of the need for updated reference ranges comes in part from the suggestion that ultrasound-imaging technology has improved. This has resulted in better lateral resolution and improved endocardial visualisation with the introduction of double harmonics. As a result, data collection from more diverse and heterogeneous samples could also lead to changes in RV dimensions. It is also possible that the culture of RV assessment will have changed over time, such that a more in-depth assessment is now carried out routinely.

Therefore, the belief that the RV remains the poor relation to the LV in terms of assessment via ultrasound is redundant and the adoption of guidelines pertaining to the assessment of the RV alone is an important step in creating thorough chamber quantification.

4.2.1. Gender difference in Linear dimensions

Studies reporting differences according to gender in linear dimensions have been demonstrated by both echocardiography and cMRI studies measuring both LV and RV size (Tamborini et al., 2010, Maceira et al., 2006a, Maceira et al., 2006b, Willis et al., D'Oronzio et al., 2012). Simple t-test analysis of the uncorrected linear data shown in Table 3.41 confirmed that male results exceeded that of females in nine of the ten measurements across both apical and parasternal images. The regression analysis shown in Table 3.7 – 3.9, however, which included additional factors such as height, body mass and ethnicity, found differences ($p < 0.05$) according to gender when corrected, in only 50% of the acquired measurements.

Specifically, gender differences were apparent within all RVOT measurements with the measurement in males exceeding those in females by 1.2 to 2.4mm and this was associated with small but significant increases with body mass (0.08mm to 0.1mm/kg $p < 0.0001$). In addition to the RVOT measurements, RVD-1 and RVD-2 were also significantly larger in males displaying similar increases as those shown in RVOT measurements.

RVESA and RVEDA were independent of gender after controlling for additional factors. Both height and body mass were significant contributors ($p < 0.05$) with height accounting for approximately a 9mm and 7mm increase in RVESA and RVEDA respectively suggesting a reliance on body size that could remove the gender bias. The lack of differences according to gender after regression in RVD-AN is interesting. The use of this fixed-point marker within the basal segment of the RV, despite displaying significant differences with initial testing now presents as gender-independent after accounting for body size.

One potential reason may be that the annular ring that secures the TV does not increase in size under the same conditions as the RVOT or RV muscular dimensions. Despite the adjustment in 2010 guidelines to a "greater than" value, the results of this study suggest that current recommended RV measurements may still under-represent the true size of the normal RV. Careful consideration was given to the optimised angle of RV measurements to obtain the largest dimension without foreshortening the image.

The 2010 study was retrospective therefore the authors had no control over the acquisition of the images and their adherence to this maximised RV chamber size with RV measurements potentially taken from the traditional apical four chamber view in place of the optimised RV view now advocated (Rudski et al., 2010). When the upper reference limit from the guidelines was applied to both genders, individual results of both males and female volunteers were found to exceed the suggested maximum dimensions. By the nature of what we have already described, this phenomenon was more evident for male results.

The male calculated 95% upper reference limits shown in Figure 3.21, exceeded eight out of the possible nine measurements advocated by Rudski, placing a number of healthy volunteers in the abnormal category, whilst displaying lower area measurements compared to those suggested by Lang et al (Lang et al., 2005). Similar results have been demonstrated with endurance athletes, in which a degree of RV adaptation to exercise is anticipated (Oxborough et al., 2012b). These endurance athletes, however have anticipated RV changes inline with the volume and intensity of training undertaken and as such these changes are anticipated physiological adaptations to exercise they are not suffering from any pathological cause for increased RV dimensions and therefore can also be described as being 'healthy' individuals.

The potential to then assess healthy, non-endurance patients with some form of RV adaptation to exercise is also possible, and what these results suggest is that even within this group of healthy volunteers engaged in non endurance athletic activity, there remains some cardiac measurements in excess of current guidelines.

This is more evident within the male population, but also within isolated individual females within the study group. The RV guidelines, do not give a breakdown of meta-analysis results by gender and therefore it is not possible to account for any gender weighting in favour of males or females.

Despite this, the number of calculated dimensions, from the healthy volunteer study group, in excess of current guidelines, was small. For females however, though the calculated upper 95% reference value was less than the guideline cut off value in six of the recognised measurements. This resulted in a 1 – 3mm overlap between the 95% upper limits of normal, for female healthy volunteers within this group, and the upper cut-off of normal generic RV dimensions.

Although the numbers are small, the range is such that there remains potential for an already dilated RV to be encompassed within the 'normal' criteria. The use of measurements stratified by gender, which have been in use within in the context of the LV for almost a decade (Lang et al., 2005), would provide more specific and potentially clinically useful information where differences between genders are apparent.

Although the aim of this study is to standardise and quantify RV measurements, additional skill is required to ensure a false positive diagnosis is not made in cases of suspected RV dilation, purely based on RV calliper measurements. This includes visual inspection of the RV to LV ratio with the RV presenting as approximately one third of the LV size. Despite this the notion that a significant difference in male and female hearts exists in the absence of pathology is well accepted, but currently lacking in recognised RV guidelines (Rudski et al., 2010).

This suggests the need for measurements stratified by gender to be available when simple linear scaling is to be used. The implications of this need to be further investigated within conditions such as PH given the increased incidence in women (Badesch et al., 2010). Despite this, women have been shown to have an improved survival rate (Shapiro et al., 2012) which clinically may result in additional follow-up

studies to monitor the disease, increasing the importance of accurate and reproducible measurements of the RV.

Echocardiography has already been suggested to be one of the more common diagnostic tests used within this pathological field (D'Alto et al., 2013), it therefore becomes imperative that increases beyond normal linear values, specific for women, and subsequent increases in RV size can be documented accordingly. The benefits of scaling both ratiometric and allometric methods relative to the RV are discussed in sections 4.4 and 4.5.

4.2.2. Ethnicity and Linear dimensions.

Regression analysis indicates that after accounting for other confounding variables ethnicity remains a significant predictor for several RV measurements. RVOT 1 and RVOT-3 in the European group and, when compared to the reference European group, the Afro-Caribbean group (RVD-AN, RVD-1 RVEDA and RVESA), presented with the largest RV dimensions. Both basal measurements RVD-AN and RVD-1 were approximately 4.2mm and 5.2mm larger in the Afro-Caribbean group compared to the European reference and significantly larger than the remaining ethnic groups.

Extensive work has been undertaken on assessing the physical adaptation to exercise or the associated changes with exercise in athletes of varying ethnicities (Oxborough et al., 2012b, Zaidi et al., 2013). Population based studies into LV dimensions have been conducted (Chahal et al., 2010) however limited RV normal data exists on the non-athletic, ethnic populations utilising common echocardiographic techniques. It is possible that the regarded normal shape of the RV may vary between ethnic groups as a physical manifestation, with the Afro-Caribbean groups displaying wider basal and annular measurements (b=5.19mm & b=4.1mm respectively), yet no significant difference in length (RVD-3) compared to Europeans was observed. This may also account for the increased RVEDA and RVESA measurements in which the Afro-Caribbean group continued to return the largest outcomes after accounting for the confounders previously discussed.

RVOT-1 and 3 measurements were found to be the largest within the European group. Afro-Caribbean results were largest in RVOT-2, however, Zaidi et al (Zaidi et al., 2013) recently found a similar outcome with white compared to black athletes displaying increased RVOT dimensions but across all three measurements RVOT 1-3 (reported as RVOTP).

Ethnicity was confirmed to be an independent predictor within this measurement group although, as similarly discussed within this healthy group of volunteers, the variation (R^2) due to ethnicity itself was only small and therefore negates the need to sub-divide linear dimensions by ethnicity. It also suggests that beyond simple linear associations, this variation between groups could further be influenced by other factors such as body size parameters (Zaidi et al., 2013).

The results presented in Table 3.7 however support the notion that potential changes in RVOT size may in fact represent baseline ethnic differences (Zaidi et al., 2013) prior to accounting for further body size variables. This in addition supports the findings of the MESA study that also found ethnic differences in a non-athletic population (Kawut et al., 2011). Beyond the athletic population, assessment of the RVOT region is part of the assessment criteria for Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). Based on the major diagnostic criteria provided for the confirmation of ARVC (Marcus et al., 2010) 6.4% and 5.9% of the study population exceeded this value for RVOT-1 and RVOT-3 respectively, which is a similar proportion to that found in other studies (Zaidi et al., 2013).

4.2.3. Ratio Scaled (indexed) RV Measurements

Ratio metric scaling is often employed because of the relative ease with which it can be achieved. Despite this, there are schools of thought that require certain conditions to be met before the use of ratio scaled measurements can be applied. Furthermore, the most appropriate scaling variable (height, body mass, BMI or BSA) is one that has caused some discussion (Neilan et al., 2009).

The notion that body mass as a scaling factor can increase or decrease easily with time, in addition to the complex requirement to derive true fat free mass and fat mass, negated the use of both body mass and BMI as scaling variables within this study. One study, however did show that cardiac dimensions can change with increased body mass, although this particular study was undertaken using morbidly obese individuals and therefore may provide a more extreme example compared to the healthy volunteers used within this current study (Alaud-din et al., 1990).

Using both height and BSA as a scaling variable, the ratio scaled results failed to remove any ethnic based differences, across all most all measurements, with the exception of RVD-2 scaled to height.

Although it would be easier to conclude that ethnic variation in RV dimensions results simply from changes in BSA or height, this is too simplistic an approach to the complex interactions that set apart one ethnic group from another and does not account for potential environmental, (in addition to genetic) influences on cardiac dimensions (Adams et al., 1985). Given the limited range in mean values displayed in Table 3.5, (all-be-it statistically significant $p < 0.001$ across all ten measurements) it is anticipated that any variation may in fact be subtle in its effects on RV dimensions and must be interpreted within the tolerance of the measurement technique.

Initial work regarding this assessment, highlighted the benefits of indexing to BSA(Willis et al., 2012). With the addition of the Afro-Caribbean population to the study group, this further increased the number of significant (post scaling) ethnic relationships and removed any of the normalisation achieved by ratiometric scaling regarding ethnicity.

Having already established that differences according to gender existed within the simple linear results, the use of ratiometric scaling was able to produce (for linear dimensions) measurements independent of gender when scaled to height. This is not uncommon with the already well-established, exponential increase, in cardiac dimensions associated with an ensuing increase in body size (Neilan et al., 2009), although this is more prominent in the development from child to mature adult. The failure to achieve this with both EDV and ESV for the RV, is probably due to the

variance in scaling factors with height being considered one dimensional compared to the two dimensional measurement of RV volumes from standard 2D images (George et al., 2001). With this gender independent association across the linear measurements it was possible to accept the hypothesis that when accounting for body size, gender differences were no longer significant. This is further demonstrated with the more robust allometric assessment discussed in section 4.2.4.

BSA was less successful with differences according to gender remaining across RVD-3, RVOT-2, RV-WT and RVESA despite scaling to BSA. The use of the Dubois Dubois formula may have influenced the results here. Given that earlier results have established the success of height as a single ratio scaling variable, the combined use of height and body mass did not prove as enlightening. The weighting given to each of the scaling variables in the Dubois formula may have reduced in accordance with the body mass interaction, the impact of height as a single scaling variable.

These results suggest that much like the LV, RV assessment is in need of further scaled measurements, to be used in conjunction with standard measurements. Indexing alone was not sufficient to normalise all results, with significant differences still present when comparing ethnic groups (Table 3.10).

Clinically the use of RV dimensions scaled to biological parameters may provide additional prognostic information not only for the patient population but also for other groups such as endurance athletes, who have recently been shown to have absolute RV dimensions well in excess of ASE guidelines (Oxborough et al., 2012b).

Unlike BSA, age was found to contribute only minimal influence to a minority of measurements (RVD-3, RVESA and RVEDA),showing a decreasing trend with increasing age in males only. Despite similar results demonstrated with RV volumes showing a decreasing trend in both echocardiography and cMRI with age, this could also result from the smaller number of normal volunteers with increasing age in addition to the manner in which each measurement is determined using the major axis dimension (Maceira et al., 2006b).

4.2.4. Allometric Scaling

Accounting for the influence of body size with any indexed parameter should result in a low correlation with the specific scaling variable (Kawut et al., 2012). Despite the common use of BSA as a scaling variable in both LV and RV guidelines, its widespread use has been questioned for a number of years due to the lack of conformity to TSC often found (George et al., 2001) (Oxborough et al., 2012b). In addition, a recent review also called for further work to be undertaken to establish the correct scaling variables to use for the RV (Hoit, 2012).

The first stage in determining the suitability of data for allometric scaling therefore involves the calculation of the data using TSC. Previous research has suggested that the calculated dimension of the measurement will influence the conformity to TSC (George et al., 2001). This remains the case with the results displayed in Table 3.13, which indicate a lack of agreement for BSA with a partial correlation shown for height. Height as a one-dimensional scaling variable, displayed more agreement to TSC across one-dimensional RV measurements but displayed a highly significant difference with both RV EDA and ESA ($p < 0.001$). It could be argued that these both remain two-dimensional measurements and therefore subject to the same uni-dimensional association.

By comparison, measurements using the BSA x/y correlation, displayed highly significant differences across almost all RV measurements ($p < 0.001$), although this may suggest that it was a mathematical certainty that the use of BSA as a scaling variable would not to conform to TSC. The advantage of scaling data allometrically can also be visually displayed. The graph shown in figure 4.1 demonstrates the limited fit of the $y=a.x^b$ approach, compared to the allometric approach, with increasing BSA.

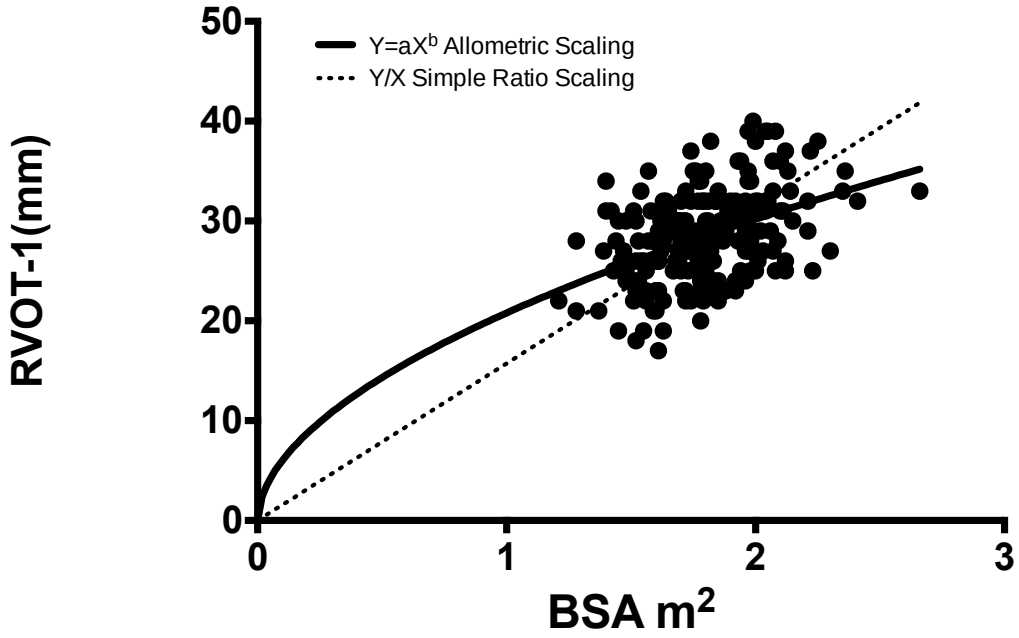

Figure 4.1 A graphical representation of ratiometric scaling, and allometric scaling

The use of height as a scaling variable has been questioned (George et al., 2001), but this criticism has tended to centre on studies of athletes in which the use of height displays a large degree of homogeneity. The advantage of the study reported in this thesis, was the large recruitment of healthy volunteers with no predisposition to certain body types. There was a tendency, however to exclude studies if the image quality was poor and as previously alluded to, this was more prevalent with increased adipose tissue.

Despite the heterogeneity of the study sample, the calculated b values listed in Table 3.14 for height either included, or were very near to 1.0 within their 95% confidence intervals. This suggests that allometric scaling would not be too dissimilar to standard ratiometric scaling which in turn suggests a more linear relationship between height and RV dimensions (George et al., 2001). It must therefore, be considered that the continued use of BSA as a scaling variable, remains clinically relevant due to the influence of height within the formula, but the addition of body mass may not add to its significance.

The use of allometric scaling provides a suitable alternative, which will better represent many of the non-linear associations, found within physiological

relationships (Dewey et al., 2008). Nonetheless, the determination of appropriate scaling factors could remain a source of variation, with each scaling factor being pertinent to the specific sample from which it is was calculated.

Collectively, studies have shown similar scaling factors, for example BSA used in the determination of LV mass has shown b exponents in the range of 1.44 to 1.67 for both normotensive subjects (de Simone et al., 1992), junior athletes (George et al., 2001) and weightlifters versus healthy controls (George et al., 1998). The variation between the b values determined from studies featuring similar groups is unlikely to change the derived reference ranges between these groups significantly, however it remains imperative that any additional associated scaled factors are documented.

A recent study into the scaling of athlete RV dimensions demonstrated the clinical use of this technique for stratifying above average, clinically normal athletes (Oxborough et al., 2012b). The b values obtained in that investigation, when applied to the population within this study, did produce significantly different ($p < 0.001$) results for the heterogeneous healthy population featured within this study. The calculated scaled RV dimensions, RVOT-1, RVD-1, RVD-3 and RVEDA were all significantly larger using the athlete derived scaling formula compared to the b exponent derived from the healthy, non-athletic population.

Caution must be exercised when using b values derived from other studies. Although the difference between these two calculated samples remained small, the mean values (RVOT-1 20.6+2.8mm vs. 23.7+3.3mm; RVD-1, 21.4+3.0mm vs. 24.0+3.4mm; RVD-3, 59.1+4.8mm vs. 59.7+4.9mm; and RVEDA, 7.4+1.8cm2 vs. 9.7+2.3cm2) are nonetheless clinically significant.

Potential bias can be minimised with good sample size and recruitment providing a sample mean representative of the population mean. In addition, the inclusion of additional scaling factors will adjust the calculated b value. Rational assessment of the clinical applicability needs to be made to ensure disparity within subject measurements is minimised allowing the technique to provide well-adjusted measurements.

It is beyond the scope of this study, and clinically impractical, to investigate and extrapolate a large number of possible b exponents to derive normal values. The RV measurements both ratio-scaled and allometrically scaled, within this population are therefore unique to this study.

It is clear from the data that has been presented that in the case of BSA, size independence was not achieved and therefore the use of this as a reliable method for assessment may potentially be flawed (Oxborough et al., 2012b). The use of allometric scaling is not in daily clinical practice, however and therefore the use of BSA as an initial indexing factor should be considered as a minimum when appropriate scaling data is not available.

The results of this study provide b exponents for each of the ten suggested RV dimensions which when used to construct scaled indices, provide body size independent measurements. We believe this is the first time this has been done in a heterogeneous group of healthy, non-athletic individuals, and provides a valuable starting point for further work to be undertaken in order to advance the clinical usefulness of linear measurements.

By effectively removing this body size dependency, it became possible to further explore the relationship between ethnic groups and the association with RV size.

4.2.5. Ethnicity and allometric scaled dimensions

In order to account for the most accurate RV assessment method, further allometric scaling of RV dimensions was undertaken with the addition of ethnicity into the equation. The impact of body size in calculating normal LV dimensions is well established, with European guidelines advocating the use of BSA (simple scaling) for male and female dimensions (Lang et al., 2005).

With the inclusion of an Afro-Caribbean cohort, further assessment allowed investigation into the potential effect ethnicity might have on cardiac dimensions relative to body size. The associated body surface area and ethnicity (b and c

exponents) derived from the allometric scaling are shown in Table 3.22. These suggest that ethnicity has only a very small effect on cardiac chamber size relative to body surface area resulting in little effect on the calculated b values shown in Table 3.14. Interestingly though, the results of both ANOVA and regression analysis (Table 3.18 -3.20) still highlight significant differences between ethnic groups post scaling, suggesting that ethnicity may have an impact on RV dimensions, but this is not related to body size.

The European and Afro-Caribbean groups demonstrated larger RVOT measurements compared to Indian, Chinese and Malay groups, independent of both gender and body size. It would be pertinent to acknowledge these differences between these ethnic groups when comparing results that have been scaled using the allometric variables calculated from this study. These particular results have demonstrated a similar finding to those in other recent work undertaken on both black and white athletes (Zaidi et al., 2013), with European RVOT-1 measurements exceeding that of the remaining groups ($p < 0.001$), but without the suspected influence of athletic training and within a group independent of body size.

The 95% upper reference values displayed in Table 3.18 display the maximum measurements derived for each ethnic group. Alternatively, Table 4.0 provides URV measurements for the study cohort. With the exception of RVD-2, in which Chinese measurements were the largest but non-significant (p ns), Table 3.18 suggests that either the European or Afro-Caribbean groups account for the largest measured values exceeding those of the Malay Indian and Chinese groups.

In addition to the statistical significance found between these groups the established measurement variability discussed in section 4.3 regarding RV measurements should be considered for those that fall at the upper extremities of any 95% reference value and should be interpreted within the clinical context of the patient.

Therefore, in response to the primary aim of this study that ethnicity will influence RV dimensions, we can accept this with the suggestion that these results may represent baseline ethnic differences (Kawut et al., 2011) but, as previously discussed, this is minimal, not consistent across the full RV assessment profile and most likely

not related to body size. In agreement, this would indicate that for practical reasons, the use of RV dimensions organised exclusively by ethnicity would be unnecessary (Zaidi et al., 2013) and factors beyond self defined ethnicity such as environmental factors (Adams et al., 1985) must be considered in greater detail and with a wider range of pathological conditions. Comparisons of European and Afro-Caribbean RV dimensions with other ethnic groups, however should acknowledge the potential for ethnic influence within their results.

Genetic influence on the size and potential for adaptation of cardiac chambers is beyond the scope of this study. It is clear that despite recognised differences in both physical and cardiac size at baseline, when this is accounted for between groups, variations between same sex ethnic groups can be demonstrated. The relative effect on RV dimensions, as a source of error, within this study are likely to be limited given the restricted measurement technique compared to detailed genetic mapping. Despite previous attempts to identify the cardiac adaptations to exercise using genetically identical twins, Adams et al (1985) concluded that both genetic and environmental factors were key determinants in cardiac adaptation.

This study aimed to also test the hypothesis that allometric scaling is more effective at producing body size independent measurements compared to ratiometric scaling alone. The combination of results described within this section demonstrates both the evidence for and the effects of allometric scaling, allowing us to accept this hypothesis with the recommendation that allometric methods be employed in those patients in whom a high clinical suspicion of abnormal RV measurements or in those who may present with increased BSA.

This approach is further supported by the reference values provided without any associated scaling (ratiometric all allometric) and as with all LV assessment this will remain the first line of clinical assessment with a step-up approach to body size measurements if required.

4.2.6. RV dimensions and Age

The limited associations between age and RV dimensions displayed within the raw data in Tables 3.7 – 3.9, suggest that the inclusion of body size parameters, gender and ethnicity account for a higher proportion of any measurement variation by comparison. Despite this, it is possible to accept the study hypothesis that increasing age will lead to altered RV dimensions although this relationship displays a biphasic response depending on the location of the measurement.

When both body mass and height are accounted for with their respective allometric indices (Tables 3.15 – 3.17 and 3.19 to 3.21), the statistical significance of age is amplified across age groups, specifically within the parasternal measurements displaying an increase in RV size with increasing age. By comparison, area measurements significantly decrease, whilst apical measurements display a tendency to decrease in size also (p= ns).

This disparity between age related measurements obtained from different locations within the RV may result from the compartmentalisation that is recognised to exist within the RV as previously discussed. With the reduction in RV volumes shown in Table 3.30, and the trend in both apical 2D measurements and RV area measurements suggested to occur with increasing age, the potential for RVOT measurements to increase with age may represent a compartmental equilibrium across the RV. This action potentially accounting for the reduction in RV size often expressed from within the apical window (Henein et al., 2014) .

The lack of any significant correlation between PAPSP and age (p=ns) suggests that this is not in response to raised systolic pressures within this group. The influence of age on global RV diastolic function however, as demonstrated in Table 3.27 with STE SRe and SRa, may impact on this more mobile portion of the RV resulting in increased dimensions within this body size independent group. This interaction is complicated further by the notion that the RV body itself generates the majority of RV filling, therefore further work is required to ascertain the impact of the regionalised RV.

4.2.7. Summary

Although the results of this study have demonstrated allometric scaling as an alternative to ratiometric scaling, through the biological flaws of a non-linear relationship, it is anticipated that BSA will continue to be used in clinical practice. The most common approach is to index (by any means) dimensions that fall outside or at the upper limits of normal measurements.

The decision to further scale cardiac dimensions is judged, in part, by both clinical skill and the calculated linear measurement. As a result, simple linear measurements are likely to remain in common use. This suggests the need for several different normal dataset ranges that could lead to further confusion, rather than more standardised reporting.

Interpretation of the calculated cardiac information available remains the responsibility of both the sonographer and governing bodies. Based on the information within current guidelines for RV assessment, measurements based on gender are required. In addition, the use of allometrically scaled BSA measurements should be employed, since this has been shown to remove the influence of gender and thus provide a size and gender independent range.

4.3. Repeatability Results

Quantification of chamber size and function remains one of the common referral requests in echocardiography (Lang et al., 2005). As a result, this will inevitably involve for patients repeat scans across a range of operators. Both operator skill, consensus on measurement location and appropriate training will all improve reproducibility (Evangelista et al., 2008).

This formed the basis for the protocol for RV assessment within this study. It is recognised that reproducibility will vary between different methodologies as well as operators (Evangelista et al., 2008) therefore each of the methods used to quantify RV size and function are reported independently. Acceptable levels of agreement and

repeatability were found within the RV across most of these measurements, particularly measurements made within the apical views.

It is not possible to say categorically that the standardisation of these measurements improved repeat testing however it is demonstrated that despite a change in operator, acceptable measurements can still be obtained using this technique. Notwithstanding the increased measurement variability COV and lower reliability ICC scores, RVD-2 is an important measurement given its use to quantify the RV minor axis when the apex is poorly visualised or in cases where specific segmental changes to RV anatomy could occur such as ARVC (Gemayel et al., 2001).

These results suggest that there may be an increased technical or interpretation challenge in calculating this measurement regarding the placement of the calliper measurements. The confusion surrounding the placement of this measurement has already been documented (Valsangiacomo Buechel and Mertens, 2012) with Lang et al (2005) referring to measurements made at the LV papillary muscle level whilst Foale et al referring to it as being made in RV body (Foale et al., 1986).

Although Foale provided the original reference source, this description could leave the measurement open to interpretation between sonographer resulting in the increased measurement variability (COV 21%) and interobserver measurements, shown in Table 3.23. Re-test analysis, however, indicated improved measurement variability suggesting that for this particular measurement, interpretation by the sonographer is potentially a significant factor in determining RVD-2 size.

A greater degree of variability was found with the end-systolic area measurements, which, as previously noted, is most likely due to the densely packed trabeculated fibres within the small cavity size limiting endocardial definition (van der Zwaan et al., 2011b). Subtle changes in the orientation between images may account for some variability in agreement and repeatability scores for both the short and long axis parasternal images. Despite this, acceptable levels of agreement were still achievable within these commonly used views. Given the little variation in the measurements made of the RV-WT (range 0.3-0.6 cm mean 0.4 cm) a small difference in repeat measurements would result in a large calculated variability.

Given the complex nature of RV geometry and the difficulty in repeating consistent imaging planes we have demonstrated that, out of all measurements, RVD-AN, RVD-2, RVD-3, RVOT-1, RVOT-2 and RVOT-3 are the most consistent.

Measurement variation seems to be concentrated around two main factors, the user and the image. Different users measuring the same image account for a similar variation as the same user measuring two images of the same subject (van der Zwaan et al., 2011b). This suggests the potential source of variation comes from the individual interpretation of where each measurement should be made. Locating the exact insertion point of the LV papillary muscle proved challenging and in many cases did not measure the widest body of the RV.

TAPSE proved the easiest method of RV functional assessment, whilst FAC encompassed some of the error derived from the area measurements expressed earlier. Despite this, the use of FAC to quantify RV systolic function should not be underestimated. FAC provides the only 2D method from which systolic and diastolic area changes can be directly assessed, unlike TAPSE and RV S' which provide a surrogate of total RV function.

Utilising additional techniques such as contrast enhancement could help to improve the lateral wall border delineation however the practical and cost applications of this for a routine study would need to be further evaluated.

4.4. Speckle Tracking Echocardiography

STE is a new modality designed to provide quantitative information unlike many other traditional cardiac functional measures, by assessing the deformation of the myocardium directly. Changes in LV deformation have been extensively studied with results closely linked with poor clinical outcomes and changes in myocardial mechanics (Cimino et al., 2013). Although studies into normal RV deformation characteristics are limited, despite this STE measured RV strain has been shown to

predict deterioration of the RV in adult patients with pulmonary hypertension (Sachdev et al., 2011).

Attainment of strain images within this study was similar to that found in previous LV adult studies, (Dalen et al., 2010) (Marwick et al., 2009) with acquisition possible in 71% of the participants. Although as a group, these were notably lower in the Malay population. The reasons for this have been discussed previously but what this demonstrates is the reliance of STE as a technique, on the quality of the 2D images taken as a baseline.

Attainment of STE data within this study, may appear low in comparison to some studies, however patients were not recruited based on initial image quality, in attempt to replicate recruitment or application in normal clinical practice. In addition, this also served to establish a reasonable perspective on the repeatability given the range in image quality.

Global peak systolic strain (GPSS) showed a weak significant negative correlation with traditional 2D measurements of RV function TAPSE, TDi. Similar results were found in a study of 100 normal patients (Meris et al., 2010). STE assesses myocardial function in a different manner to TAPSE, TDI and FAC. Whilst the correlation is encouraging, it is unsurprising given the nature of the measurement methodology. STE measures the change in speed and deformation of the myocardium rather than the change in volume as a surrogate of systolic function.

This direct assessment could be influenced by the unique way the RV pattern of contraction occurs in comparison to the LV. These correlations suggest that GPSS may be considered a more independent predictor of overall RV function given the more inclusive nature of the assessment. STE has already been shown not to be related to body size and therefore it is not considered necessary to scale results to any particular body parameter (Oxborough et al., 2012b).

4.4.1. Global vs. RV Freewall assessment.

Variations in tracking quality have previously been identified in the LV with the septal region demonstrating the highest levels of software-identified tracking coherence (Marwick et al., 2009). This further enforces the suggestion that assessment of the RV freewall using STE provides clinically alternative data compared to that derived from the global approach, which cannot reliably exclude the influence of LV function within the septum. Despite this, there remains a place for the global approach given the improved reproducibility shown within this study.

The results in Table 3.42, including the septum as part of the global RV analysis, resulted in reduced (less negative) mean global systolic strain and strain rate values compared to the RV freewall, as well as significantly lower early and late diastolic measurements ($p<0.001$ respectively).

Partitioning of the LV and RV septum is difficult both anatomically and with 2D imaging. This is due in part to the inability to differentiate a dominant left or right side leaving the septum to be regarded as a stand-alone structure. The musculature of which contains the same fibre structure as the LV free wall (Buckberg, 2006). This fibre structure may account for the age related changes noted in global diastolic strain measurements but a reduced association for RV freewall measurements.

At rest, and in the disease free heart, this may not result in any undue changes to the contractile function of the chamber with the ventricular interdependence relationship remaining intact. The progression of conditions such as PH, however, could alter this relationship resulting in changes to septal function due to increased pulmonary pressure. Assessment of the pure RV freewall although not traditionally global in its nature, should allow for a more unbiased assessment of one component of RV function and with further work, could provide information regarding early stage RV failure prior to the development of left-sided disease.

Despite the increased interest in this approach, only limited normal data on both systolic and diastolic function exists. In addition, the correct reporting and standardisation of assessment is important for any future work. Given that changes to

the region-of-interest can affect the reproducibility of strain results (Marwick et al., 2009), the use of RV specific tracking software could further enhance the diagnostic power of this technique.

The use of the RV freewall approach does limit the global assessment of the RV to only three regional areas and therefore like both TAPSE and RV-TDI is providing a reduced surrogate of total RV function. Despite this, recent studies have utilised this lateral wall only technique in both normal and various pathological groups with good results (Tong et al., 2008, Oxborough et al., 2012a, Fukuda et al., 2011, Teske et al., 2009b, Meris et al., 2010).

4.4.2. Age related changes

Compared to the global regression shown in Table 3.43, RV freewall regression (see Table 3.43) displayed no association between systolic strain or strain rate data and age. Other studies have also demonstrated this age independent association between strain and strain rate (Tong et al., 2008).

Regression results shown in Table 3.26 demonstrated only minimal age related changes in diastolic function, which in turn was limited only to the late (SRa) strain rate period. As aging occurs across the basal mid and apical septum, and normal, age related diastolic changes are demonstrated within the LV; aging and diastolic changes in the RV freewall may not occur at the same rate.

Similar age related, late diastolic findings have been documented with segmental RV freewall correlation to age in addition to a weak but significant correlation with the early diastolic phase (Tong et al., 2008). The study sample age was similar within this particular study (Tong et al., 2008) with a mean age of 38.49$\pm$12.76 years (n=75) compared to results from this thesis: 40$\pm$11 years (n=190).

This may result from changes in the myocardial content, with a predominance of longitudinal fibres and thinner walls compared to the LV. In addition, the lower pressure system may tolerate changes in RA filling patterns better. There remains a

lack of knowledge about the diastolic function of the lateral wall alone and further work needs to be undertaken to establish specifics of aging based RV mechanics.

One theory suggests that the RV is subject to an aging cardiomyopathy which is characterised by a loss and quality reduction of myocytes found only in male myocardium (Olivetti et al., 1995). A reduction in the myocyte elastic recoil, resulting in a replacement by fibrotic stiffening, could result in the increasing level of diastolic changes observed within the study sample (Kawut et al., 2011). This is common, however and regarded as part of the normal aging process. Further work with older patients is required to establish the differential risk in the aging population

4.4.3. Ethnic variation.

Despite there being no global changes in ethnic strain values (both systolic and diastolic) after adjustment for gender, age, height, body mass, and heart rate, differences in both RV-freewall SR and SRa were noted between the Afro-Caribbean group and the reference European group, as shown in Table 3.43.

Results from the large epidemiological MESA group have found both LV strain (Fernandes et al., 2011) and RV function (Kawut et al., 2011) results similar to those found within this study involving both African-American and US census defined black volunteers. These changes however, despite being statistically significant, are potentially subclinical within this normal group given the small-unstandardized beta values.

Despite this, the notion that there are ethnic differences in RV myocardial function, could become more evident with advances in early stage disease assessment or within other well groups that have demonstrated some form of cardiac adaptation. For example, within select groups of athletes, ethnic and gender differences in RV dimensions have already been established (Zaidi et al., 2013)

4.4.4. Gender

LV based studies have already demonstrated the significance of gender on strain measurements (Dalen et al., 2010) (Fernandes et al., 2011) though normal reference values for different genders, for the RV are limited. The results of the HUNT study demonstrated consistently higher LV strain levels for women in their large study group of healthy volunteers (Dalen et al., 2010). Similar strain results have also been demonstrated with MRI tagging studies (Lawton et al., 2011). The increased RV freewall values seen in women demonstrate similar changes seen with LV ejection fractions, in which women also exceed men (Roeters van Lennep et al., 2002).

This difference in contractility is often attributed to the cardiac protection of hormones such as estrogens, which help to reduce cardiac risk (Roeters van Lennep et al., 2002) and by the mechanics of smaller chamber volume size. A similar gender finding in both the global and the RV freewall affirms the need for gender stratified normal measurements of RV strain and in addition may demonstrate baseline contractile differences in gender which may help explain the difference in cardiovascular disease (Lawton et al., 2011). These must be interpreted with caution however since calculated 95% reference values result in a wide reference range potentially reducing the clinical usefulness of these measurements within normal clinical practice.

The use of an RV freewall PSS normal cut-off value of -18.5% for females and -17.5% for males and strain rate values of -1.14 1/s for females and -0.91 1/s for males, can provide an initial figure based on this large normal group and stratified for gender. This echoes similar global results from a study featuring both healthy controls (n=100) and patients with RV dysfunction (n=76) who found a PSS cut-off value of -19% was helpful in distinguishing between these two groups (Meris et al., 2010).

The remaining covariants such as HR require further investigation. The contribution (in relative terms) to the current model of body mass, was small and difficult to stratify clinically, however the notion that increased weight results in lower myocardial strain (Barbosa et al., 2013, Labombarda et al., 2013) and changes in RV morphology have been documented in both overweight and obese groups (Chahal et

al., 2012a). This has important future implications for levels of obesity and the link to cardiovascular disease (Shah et al., 2013).

4.4.5. Segmental results.

Speckle tracking analysis using a segmental approach has been reported in both LV and RV studies (Dalen et al., 2010, Tong et al., 2008, Meris et al., 2010). Differences according to gender, in regional strain results are likely to result from the same functional cause as both RV freewall and global PSS. With increased regional deformation, this has been attributed to hormonal levels increasing contractility.

The associations found between gender and ethnicity with both the global and RV freewall approach, are not so easily identifiable within the segmental results. These localised differences, although statistically significant, may be influenced by the increased measurement variation within each group represented by increased standard deviations.

Segmental repeatability displayed varied results across the three test scenarios ranging from acceptable to very poor. The HUNT study looked to assess and define normal segmental strain within the LV and also found higher COV scores compared to global assessment (Dalen et al., 2010). In addition, both the image acquisition and regional calculation resulted in a high degree of measurement variability and poor reliability, as demonstrated in the interobserver and test retest analysis. These poor scores limit the usefulness of these segmental results for clinical application given the large degree of variation around the mean.

It should be noted that the use of STE requires experience in both the acquisition and interpretation of images and subtle changes in the orientation of the image, which may not be obvious at the time of acquisition, or in terms of caliper of area measurements, may have resulted in the poor reproducibility statistics described in section 3.8.6. Furthermore, the effects of the manufacturer's LV specific algorithm used in the study are also not to be estimated. Indeed, recent studies have identified vendor differences as an issue surrounding the technique (Nelson et al., 2012) and this

has resulted in the pursuit of a generic, vendor independent, algorithm that will work across a number of software platforms. Overall, it was not appropriate to define normal segmental reference ranges and any subsequent associations with ethnicity, age or gender have to be interpreted with caution.

4.5. Repeatability

Test-retest analysis and results assessing repeatability within STE measurements of ventricular function are limited in LV studies (Jenkins et al., 2004) and this appears also to be the case for RV studies. This study provided a robust assessment of both intra and interobserver variability and repeat acquisition (test-retest) with a second set of images acquired by a second sonographer. One reader representing the 'best case scenario' in clinical practice then conducted an assessment.

The inter and intraobserver results showed acceptable levels of agreement across all four measurements with lower ICC scores found within the SR retest data. A recent study also showed reduced levels of agreement with RV SR parameters (Oxborough et al., 2012a). This has been attributed to the poor signal to noise ratio experienced with SR measurements that themselves are determined by arithmetic adjustment of strain values.

RV freewall results had reduced ICC scores compared to global analysis in all three test scenarios. This may result from the difficulties faced in defining endocardial and epicardial borders on the thinner more mobile RV freewall, in addition to the potential limitations of using software designed to track and follow the LV. The results of this study suggest that small changes in deformation characteristics should be clinically interpreted within the limitations of agreement displayed.

Unlike global results, which are calculated using a vendor specific algorithm over six segments, RV freewall measurements may still be subject to the effects of segmental

analysis given the modified assessment, which is less favourable in terms of repeatability (Thorstensen et al., 2010).

4.6. Base to apex gradient

Several studies have reported the potential for a gradient to exist in PSS measurements, between the basal portion of the heart and the apex, referred to as the base to apex gradient. The data presented in Table 3.28 indicate that the segmental gradient exists for both males and females, as a decreasing basal (-27.24% & -29.04%) to apex (-24.66 & -26.88%) gradient within the lateral wall in one direction and as an increasing gradient from basal (-17.80% & -19.05%) to apex (-18.07% & -21.43%) for males and females respectively, in the septal regions.

One suggestion for the alternating gradient is the result of ventricular torsion and the attachment of the crista supraventricularis (CSV) in the contraction and squeeze of the RV as part of the ventricular interdependence relationship (James, 1985). As the apex rotates in a counter-clockwise direction and increases tension on the CSV, this could result in a more pronounced pulling motion in a lateral direction compared to the longitudinal displacement shown in the basal segment, resulting in increased (more negative) PSS values in the basal lateral wall compared to the apical lateral.

There appears to be a discrepancy regarding the direction of the base to apex gradient within the RV freewall (Teske et al., 2009b, Teske et al., 2008, Tong et al., 2008, Meris et al., 2010). Importantly however, the poor reproducibility statistics and increased standard deviations derived from this segmental study, compared to both RV freewall and GPSS, might lead us to question the usefulness of this reported gradient that has been described in the literature.

Whilst the anatomical considerations are correct surrounding the mobility of the RV base (tricuspid annulus) versus the RV apex, the poor reproducibility and agreement demonstrated in this study for segmental analysis, suggest that this should be

interpreted with caution since its clinical significance has yet to be proven and the suggestion of a gradient may in fact result from the measurement tolerance itself.

The results of the retest analysis demonstrated this with the introduction of a repeat scan with a second sonographer for comparison. Even with the operator remaining constant, the angle of acquisition and interpretation of the endocardial and epicardial borders were such that the resulting segmental analysis showed a reduced level of agreement between scans. If studies demonstrating intraobserver and interobserver analysis are limited for the RV, retest analysis is even more limited (Teske et al., 2008). As a result future application of STE, ideally designed with the RV as the primary chamber, within routine clinical practice must consider the usefulness and clinical impact of the segmental approach in addition either global or RV freewall.

4.7.3D RV Assessment

This study was successful in prospectively acquiring a large and diverse 3D dataset on a group of healthy volunteers. The findings are consistent with those of earlier studies in healthy subjects. Acquisition was successful in 71% of the study group. This is highly likely to be related to the quality of the 2D images acquired within the initial phase of the study, and as such would have posed a more interesting observation if the 3D acquisition rates had been higher. Although this acquisition rate remains lower than that obtained in the recent study of Maffessanti et al (2013) recruitment to that study was not varied by ethnicity and as we has been shown above in section 4.1 attaining good image quality was more challenging in certain ethnic groups.

Particular attention was devoted in this study to the associations between 3D volumes and body size. It was hoped that any significant associations with ethnicity would be revealed by using robust techniques to try to remove many of the confounding factors that have commonly been associated with influencing cardiac dimensions.

The results of the 3D analysis again demonstrated, much like the 2D assessment, the incomplete association that ratiometric scaling to BSA makes within RV dimensions.

Unlike 2D calliper measurements scaled to height, 3D volumes can be considered multi-factorial. Despite the suggestion that the scaling relationship between BSA and RV volumes is not dimensionally consistent (Maffessanti et al., 2013), the scaling factors (beta values) derived from using allometric processes for BSA in relation to ESV, EDV, and SV (1.51, 1.36 and 1.23 respectively) were closer to 1.0 than those calculated for height and therefore represent the best use of indexing variables. Figures 3.5 to 3.7 demonstrate the near linear relationship between BSA and RV volumes demonstrated within this study compared to those for height in figures 3.8 to 3.10.

The BSA, allometric scaled, results shown in Table 3.35 compare favourably to previous study results in healthy volunteers (Kjaergaard et al., 2006b) and the current 2010 guidelines that present URV, indexed to BSA, in the region of 89ml/m^2 and 45ml/m^2 for RV EDV and ESV respectively despite not being organised by gender. Results were obtained from 4-5 pooled studies totalling 426 and 394 healthy control subjects for EDV and ESV respectively. Despite lacking separate results for males and females it was suggested that female results would be between 10-15% lower than those in men (Rudski et al., 2010).

When compared, the correlation coefficients of 3D parameters with age and body size derived from this study (see Table 3.30) demonstrated very similar results to those of Kawut et al (2011) and Maffessanti et al (2013) who also investigated age sex and body size measurements on 3D volumes. Maffessanti et al (2013) aimed to establish normal equations for use in determining reference values for RV volumes. These were further assessed by applying formulae calculated from cMRI (Kawut et al., 2011) in order to compare results using the same study group. The results demonstrated a significant and constant underestimation of 3D volumes versus cMRI, which led to the suggestion that in fact, these two methodologies should be treated separately and acknowledge the independent reference ranges required for each (Maffessanti et al., 2013).

Measurement differences in relation to gender remain apparent within this field of measurement. Table 3.28 shows the results of this analysis in terms of gender with females displaying smaller 3D ESV and EDV RV volumes than males ($p < 0.001$

respectively). As a result, ejection fraction is increased in females to 55% versus 52% ($p < 0.05$). The pattern of increased contractility and functional ability seen across both 2D and 3D measures within this study, suggests that the importance of measurements stratified by gender should not be underestimated. Table 3.36 presents both the uncorrected and scaled (for BSA) 95% upper reference measurements for both male and female results which currently do not feature within RV guidelines (Rudski et al., 2010). As a result, the hypothesis stating that accounting for body size will lead to gender independent 3D RV volumes must be rejected with the advice that RV 3D assessments remain gender dependant.

4.7.1. Age

The results of the multiple regression analysis, shown in tables 3.31 indicate a decrease in RV ESV EDV and SV compared to the reference age group (≤29 years). Due to the small numbers in the advanced age groups, it would be unwise to organise reference ranges by decades and therefore caution must be exercised when applying this data in the elderly. This also includes the limitations in recruiting 'healthy' older subjects (Maffessanti et al., 2013). The unadjusted results shown in Table 3.33 however, support the presence of age related changes, (in volunteers >40years of age), that have been found in other RV studies (Maceira et al., 2006b, Tamborini et al., 2010, Kjaergaard et al., 2006b).

Unlike the results from (Maffessanti et al., 2013), RV volumes were scaled using appropriate allometric process, and despite being independent of body size, they still limited associations, with a decrease in advanced age. Both the beta association and significance varied across 3D measurements with EDV indexed to BSA displaying a consistent decreasing trend compared to the reference (≤29 years) age group. Kawut et al (2011) found similar results with cMRI results showing an age related decrease in RV EDV when adjusted for age, sex and race/ethnicity.

Kawut commented on a study by (Olivetti et al., 1995) which demonstrated a process termed ageing cardiomyopathy with a loss in myocyte volume and quality which was

found to be more prevalent within males, with females maintaining a similar degree of RV mass (Olivetti et al., 1995). This fibrotic process could explain the finding of smaller RV volumes with increasing age (>40 years), although unlike in Kawut et al this was not associated with an increase in RV EF.

The number of older volunteers within this study may not have been sufficiently large to detect the anticipated increase in EF. However the suggestion that this process results from a loss of myocytes, leading to myocardial stiffening is consistent with the significant age related changes in diastolic strain rate noted with STE, as displayed in Figure 3.40 and discussed in section 4.4.2

4.7.2. Ethnicity

The association between cardiac dimensions and ethnicity has been demonstrated in a number of studies using a range of modalities (Natori, 2006, Fernandes et al., 2011, Kawut et al., 2011). It is believed that this is the first study to investigate the effect of ethnicity and several other common variables play on echocardiographic 3D RV data derived from a healthy population. Despite the results of the ANOVA suggesting a significant difference between European and Afro-Caribbean results, the associations made with additional factors such as gender, body size, body mass and age were sufficient to reduce this ethnic difference to a non-significant level.

With the suggestion that body structure may be the determinate factor in creating this ethnic association, the use of ratiometric or allometric measurements was a feasible method of assessment. The 3D data did not conform to TSC (shown in Table 3.31) and therefore was not suitable for ratiometric scaling. Previous research into 3D volumes in a group of healthy volunteers has also demonstrated a significant association between RV volumes and demographic and anthropometric data, also prescribing scaled 3D volumes (Maffessanti et al., 2013).

Further analysis of the study data was conducted to assess the impact of ethnicity on RV volumes, independent of body size. This revealed small c values between 0.03

and 0.05 that indicate, similar to the linear measurements discussed in section 4.2.5, that the additional impact of ethnicity on 3D volumes was largely independent of body size or height. These however are small and of limited value within the context of this study.

Interestingly the results displaying increased RV EDV volumes within Afro-Caribbean volunteers are in direct contradiction to similar results obtained within one element of the MESA study (Kawut et al., 2011). The MESA results identified that RVEDV differed with age, sex and ethnicity compared to the Caucasian reference group however, the African-American men and Chinese American men both displayed smaller RVEDV whilst females showed no significant difference. These results were derived from cMRI studies and this may have influenced the results given the known disparity in calculated volumes between echo and cMRI studies (Maffessanti et al., 2013, van der Zwaan et al., 2010). In addition, the volunteer recruitment will probably have featured different ethnic or racial groups and the suggestion that these results are exclusively interchangeable may therefore be flawed.

4.7.3. Image acquisition

With its thin walls, the RV presents a challenge within imaging for many modalities. By modifying the apical four chamber view it was possible to present more of the lateral wall within the axial resolution which is believed to improve 3D image quality (van der Zwaan et al., 2010). As expected, this did not then exceed 2D acquisition levels within the study group, further confounding the need for quality 2D imaging prior to 3D.

The advantage of 3D echocardiography over that of standard 2D imaging is the ability to image the right heart off axis, with the opportunity to orientate the image within the 3D software package during post processing (Sugeng et al., 2010). This reduces the risk of foreshortening and may explain why there were less significant differences between ethnic groups in the baseline unadjusted volume data, compared to the same data type derived from the 2D calliper measurements. Image acquisitions were

identified as a possible cause of the measurement variation with the Malay population displaying limited apical measurements (see Table 3.3).

This notion does present a slight paradox however, in that the acquisition of the 3D data was reduced because of the 2D image quality. Although the capabilities of the 3D dataset are reported as able to overcome anatomical problems, this does not improve the clarity of the images required to make the volume measurement taken at source. The studies that were suitable for 3D post-processing may subsequently be presented with the opportunity to be orientated in such a way not previously possible with 2D calliper measurements and thus present bigger measurements relative to those achieved by 2D area methods.

4.8. Reproducibility and Acquisition

We have already demonstrated the reproducibility of several common techniques used for assessing RV size and function. As with many newer techniques, the expectation is that there may be a steep learning curve surrounding the use of 3D volumes. Despite a familiarisation period prior to the start of the study, it is possible that acquisition improved with multiple scans and thus enhanced the repeatability within the latter part of the study. Results were available for 72% of the total sample whereas both Maffessanti et al (2013) and Kjaergaard et al (2006b) obtaining acquisitions results >80% in healthy volunteers.

Despite this, there appears to remain an impartial observational correlation between the quality of the 2D images (with a considerably smaller learning curve) and the acquisition of the 3D dataset used to calculate RV volumes. This would be anticipated given that the acquisition method requires suitable 2D images to be obtained prior to the collection of a 3D dataset.

As noted with the 2D acquisitions, the image limitations were centred on the delineation of the lateral wall which proved problematic when tracing the coronal

plane and the outflow region. Compared to other systems now available, the GE VIVID 7 system allowed only limited 3D plane review prior to storing. More recent systems have the ability to display all three of the commonly used image planes (within TomTec) whilst acquiring the live image, which allows further enhancement of the image to ensure that the outflow region is optimised.

The repeatability results displayed in Table 3.37 show similar levels of measurement variability (COV) for intraobserver EDV (6% versus 6%), ESV (8% versus 11%) and EF (8% versus 6%) and also for interobserver (EDV 12% vs. 10%, ESV 13% vs. 13% and EF 11% vs. 12%) to those previously reported in recent 3D studies using both 2D and 3D echo examinations in both healthy controls (n=15) and patients with congenital heart disease (n=22) (van der Zwaan et al., 2011a).

Both of these sets of COV results are higher than those displayed by (Tamborini et al., 2010) who reported improved results across both test scenarios and in test-retest results. Mean results across the three centres employed within Maffessanti's study show similar levels of intraobserver variation for both EDV and ESV. In addition, when compared across centres in an interobserver study design, once again increased COV results similar to those found within this study are documented (Maffessanti et al., 2013).

Tamborini's results may have been influenced by a geographic patient selection bias, with the entire study population selected from a single centre consisting of hospital staff and relatives, potentially providing some degree of homogeneity between subjects. By comparison, the volunteer population discussed within this thesis is known to be diverse both ethnically and in calculated RV volumes, shown in Table 3.31, and this may have presented a further inference on results, in addition to the technical challenges faced in acquiring images already discussed.

In further support of this, Maffessanti and colleagues used the original data from Tamborini's study of 245 volunteers within their larger study of 504, recruited from three different centres within Italy. With the potential introduction of a more diverse sample from other hospital sites, repeated measurement variability increased.

Limits of agreement reported by Tamborini et al (2010) were again lower than reported here, however this is to be expected given the already established measurement variability. The distribution (across the three test scenarios) of the LOA results shown in Table 3.37 replicates that of several studies into 3D repeatability (Tamborini et al., 2010, van der Zwaan et al., 2011b, van der Zwaan et al., 2011a) with worse agreement and measurement variability, demonstrated between different users (interobserver) than when compared to separate acquisitions and or repeat measurements (intraobserver and test-retest).

Repeat assessment has several components that are known to influence the outcome. Broadly these can be broken into patient-related, acquisition related and reader-related (van der Zwaan et al., 2011b). These results suggest that the influence of the reader related component is more influential in the outcome of results, than the acquisition. Despite the potential for images to be acquired 'out-of-plane' between studies, the readers own interpretation and/or experience in assessing 3D volume images will potentially contribute to the final result.

The clinical applications must therefore, take account for this and as with the discussion of segmental strain results in section 4.5, interpret changes in EDV of <38ml, ESV <18ml and changes in EF <12% with caution when comparing repeat studies between sonographers. Maffessanti encountered similar results when assessing inter-centre reproducibility results that displayed high COV and poor LOA. This was later reduced by the introduction of a standardised assessment protocol between centres (Maffessanti et al., 2013).

4.9. Limitations

In the context of this piece of research, there may be some bias in the description received by volunteers. Efforts to allow the participants to describe their own ethnicity during the pre-scan interview ensured we recruited the desired ethnic demographic. This may however have resulted inadvertently in adding a selection bias since each volunteer would have been aware when they consented that specific ethnic groups were to be compared, and this may have influenced the information that

was provided at consent and therefore their recruitment to the study by essentially directing them to a fixed response.

The study presents the opportunity to investigate associations within several ethnic groups. The sample size recruited was not designed to account for a large number of possible cofounding variables and produce reference ranges as a result. The study has however demonstrated similar results to other investigations with an ethnic association, suggesting the possibility of minimal ethnic variation, but this is beyond the scope of cardiac imaging interpretation, and may require genetic assessment.

Studies have shown moderate to good correlations between techniques across a wide range of subjects (Kjaergaard et al., 2006a, van der Zwaan et al., 2010, van der Zwaan et al., 2011a) in addition to detailed assessment of gender and ethnic groups undertaken by the MESA study (Fernandes et al., 2007, Kawut et al., 2011, Ventetuolo et al., 2011, Natori, 2006). In particular the association between echocardiographic and cMRI assessment hsas been discussed within this study and examined extensively within the research community, with acceptable agreement between methods, regarding RV volumes.

The aim of the study was to investigate the influence that common variable within clinical practice may have on echocardiographic RV assessment. The inclusion of a cMRI scan, therefore, although an added benefit for the reproducibility arm of the study, may not in fact provide additional information surrounding echocardiographic normal reference ranges.

The system used to acquire all the 2D and 3D data was a GE Vivid 7 system that in 2014 is now surpassed by the Vivid E9 (Vivid 7 & E9, GE Medical Systems, Horten, Norway) that has been designed specifically for 4D acquisition. Improvements in the technology used to acquire 3D data have also become available within the duration of this study. This includes single beat acquisition and improved frame rates, seen with systems such as the Phillips Epiq 7 (Epiq 7, Koninklijke Philips N.V, Netherlands), enhancing temporal resolution and potentially endocardial definition.

In addition newer modalities for the assessment of RV size and function, known as knowledge based reconstruction or 3DR (Ventripoint INC, Seattle, WA, USA) have recently been trialled. Results were comparable to MRI in a study of 70 patients with tetralogy of Fallot repair (Dragulescu et al., 2012).

Briefly, the method relies on the acquisition of 2D images of the RV whilst using an additional magnetic tracking system. Using a database of RV shapes, single point plots are made from the 2D images, leading to calculation of the 3D equivalent within the magnetic 3D space (Dragulescu et al., 2012).

Despite these advances in both image clarity and methodology, the results of both the 2D and 3D assessments derived from this study were very similar to both Foale et al (1986) and Rudski et al (2010). Further work will need to be conducted to investigate the use of these improving technologies and the effects they may have on the manner in which assessment of the RV is conducted.
The strain and strain rate results derived from this study were conducted using GE EchoPac specific algorithms and therefore may only provide limited cross vendor comparisons. Limitations in both manufacturer assessment techniques and the need for vendor specific software will continue to hamper the progress of STE as a clinical technique (Nelson et al., 2012).

4.10. Statement of work performed.

The study design, acquisition of images (as reported in section 3.1), measurement, analysis and reporting of results were all been undertaken by the author. Preparation of this and subsequent manuscripts along with both conference posters, presentations and abstracts were also undertaken by the author. In addition, the author was responsible for co-ordinating studies across sites, ethical approval and organisation of funding, and honouree contracts.All measurement of 2D, strain and 3D volume data was undertaken by the author. Additional scans were analysed as part of the repeatability studies to provide interobserver statistics performed by both DA and JS.

5. Conclusion

The present study was designed to investigate prospectively a range of echocardiographic methods used to assess the RV in a multi centre study. To ensure a diverse sample, recruitment involved several different ethnic groups varying in age and gender. This resulted in the calculation of several reference ranges for 2D calliper measurements of the RV. In addition both RV deformation (strain and strain rate) and 3D volumes and ejection fraction were also calculated, and organised by gender. The result is a set of data, which together provides a comprehensive assessment of the RV from a large diverse sample.

This observational study has determined several key findings and confirmed the work of smaller studies regarding the importance of both patient anthropometric data and gender in the calculation of reference ranges and 95% upper reference values for routine clinical practice.

Demographic and anthropometric body size parameters were found to be strong independent predictors of RV size across a range of functional, dimensional and volume based measurements. Although current assessment of the LV may include measurements indexed to BSA, this is lacking in the RV.

In comparison, allometric processes have been demonstrated to be key in removing the significant influence of body size and gender and consequently, have allowed further investigation into possible ethnic variations in RV measurements in this study. Despite calculating these values for common 2D linear and area measurements and 3D derived volumes, results of the non-linear equations suggested significant ethnic differences could be accounted for within 2D and isolated 3D measurements of varying combinations.

With the influence of body size removed both European and Afro-Caribbean results displayed larger 2D measurements in various combinations, compared to the remaining groups with only limited difference between each other. Despite the isolated differences it is remains unpractical to assume reference ranges organised by

the ethnic groups within this study, given the diversity in ethnicities but also the similarities calculated with 2D URV organised by ethnicity.

Further work must be undertaken to establish both the genetic and environmental influence on cardiac size. RV dimensions scaled using the methods described which fall at the URV should be interpreted with caution considering the potential for ethnic variation.

Regarding the practical application of these results, RV assessment of both size and function, like the LV, should be stratified by gender. Many of the measurements reported displayed significant differences between male and female volunteers with female results smaller compared to males. Functional assessment of the RV using speckle tracking derived strain and strain rate should be organised and measured against gender specific reference ranges, with females displaying higher PSS strain values and 3D EF compared to males. This multi-system approach to the assessment of the RV as a whole provides a crucial baseline from which other studies can investigate the effects of disease processes on the right heart.

In order to ensure correct identification of pathologically abnormal RV's, a systematic approach to assessment must be undertaken. In addition to the basic caliper measurements, comparisons to the left heart must also be made in terms of size and function. Should standard 2D caliper measurement fall outside of the normal linear reference range then additional assessment based on subject body size should be undertaken to confirm the potential for a dilated RV. Using the reference ranges presented in Table 3.6 and 4.0 it is possible to identify and comment on measurements that fall outside of these with a more accurate interpretation of the subjects gender and body size.

Results generated from this study have identified lower reference limits for both male and female longitudinal strain and strain rate using the RV freewall approach. Ethnic variation was minimal although differences between European and Afro-Caribbean PSR and SRa were found using the RV freewall approach only. The current findings were limited but do support that of the cMRI based MESA study which also found myocardial deformation differences between ethnic groups (Fernandes et al., 2011)

Like 2D calliper measurements, 3D volumetric assessment of the RV differs between males and females and should therefore be stratified by gender. In addition, this study has demonstrated the benefits of scaling RV 3D volumes allometrically, resulting in both gender and body size independent measurements.

Age was found to affect RV measurements but there this is a minimal and biphasic response with RVOT measurements displaying isolated increases with advanced age whilst apical measurements, a decrease, supporting the evidence found with separate 3D acquisitions, which also demonstrated a decrease in RV volumes with advanced age.

Age related changes have also been identified with deformation measurements of both early and late strain rate STE assessment. Again this technique is influenced by the approach to RV assessment and therefore must be clearly identified in any future work. This study has found that the diastolic changes seen within the septal region do not coincide with similar changes seen in the RV freewall and therefore further work will need to be undertaken to assess how this may change in certain disease states and the potential for this as a marker of diastolic heart failure.

Prior to the 2010 guidelines, assessment of the RV was limited and underrepresented in relation to the LV. This study has utilised the methodology provided by the main reference source for RV assessment prior to Rudski's et al (2010) paper namley Foale et al (1986), and has demonstrated reproducibility statistics across a range of test scenarios that together present a robust appraisal of RV assessment.

2D calliper measurements will remain a primary tool for the assessment of the RV and, when utilised with the suggested anatomical markers, provide acceptable measurement variability, agreement and reliability.

STE as a technique is best reported as either RV freewall longitudinal strain or as a global assessment within the focused RV view. Further work will need to be undertaken if the true influence of the LV septum on RV global strain is to be fully understood. Future research must also define clearly which technique is being

undertaken. Segmental reproducibility was poor and therefore strain and strain rate segmental analysis within the RV has only limited assessment qualities. This poor reproducibility may further influence the potential for a base to apex gradient demonstrated, and requires future studies to utilise RV specific STE software that is vendor independent.

Right ventricular assessment has been underreported in comparison to the LV in the past. As technology has improved so has the desire to investigate and better understand the function and normal range of the RV. The findings of this study have a number of important implications for future practice.

There is, therefore, a need for the gender specific ranges that are provided here and in addition, the results have demonstrated the benefits of scaling to body size in order to further reduce disparity between genders and ethnic groups, although the notion of ethnic influence should not be discounted completely and requires further investigation.

6. References

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7. Appendix

7.1. Ethical Approval for UK sites

Bath Research Ethics Committee
Room 11, John Apley Building
Research Ethics Office
Royal United Hospital
Combe Park
Bath
BA1 3NG
Tel/Fax: 01225 825725
vanessa.bishop@ruh-bath.swest.nhs.uk
11 March 2009

Dear James

Full title of study:	Multi Ethnic Right Ventricular Assessment in the normal heart using two dimensional and three dimensional imaging and myocardial speckle tracking.
REC reference number:	09/H0101/21

Thank you for your letter of 26 February 2009, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by Chairman Dr Brian Robinson, Dr Jenny Bell and Mr Anthony Harrison.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date	
Educational Supervisor's CV		01 February 2009	
GP/Consultant Information Sheets	GPL010109	01 February 2009	
Advertisement	VP010109	03 February 2009	
Peer Review		30 January 2009	
Covering Letter		01 February 2009	
Protocol	1	03 February 2009	
Investigator CV		01 February 2009	
Application	2.0	03 February 2009	
Letter from Helen Twemlow, Head of Adult Technical Services, re: use of equipment	HTLetter	26 February 2009	
Letter from Dr Jacob Easaw re: volunteers who subsequently require care/communication with their GP	CFU010109	27 February 2009	
Response to Request for Further Information	CVL020209	26 February 2009	
Participant Consent Form: Healthy Volunteer	VCF020209	26 February 2009	
Participant Consent Form: Patient	PCF020209	26 February 2009	
Participant Information Sheet: Healthy Volunteer	VIL020209	26 February 2009	
Participant Information Sheet: Patient	PIL020209	26 February 2009	
Letter from Sponsor		27 November 2008	
Covering Letter		08 March 2009	

09/H0101/21	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Jenny Scott
Vice-Chair

7.2.Ethical approval from Gleneagles Hospital Malaysia

JPEC Joint Penang Independent Ethics Committee	Joint Penang Independent Ethics Committee, C/o Info Kinetics /Clinical Research Centre, 3rd. Level, Gleneagles Medical Centre, No. 1, Jalan Pangkor, 10050, Penang Tel: 04-2285760 Fax: 04-2285715

Our reference: 02-09-0790

24 February 2009

Dr. Rajesh Shah
Consultant cardiologist
Co-investigator
Gleneagles Medical Centre
No. 1, Jalan Pangkor,
10050, Penang

Dear Dr. Shah,

Re: Application for JPEC approval to conduct a study: Normal right ventricular measurements in a multi-ethnic population. RESEARCH PROJECT PROTOCOL NUMBER.:*nil (JPEC 02-9-0013)*

JPEC has reviewed the above application submitted by you on the 19th February 2009. There are few documents/issues that need to be put in place before a proper review of the application can take place.

JPEC complies with the standards stated in the Declaration of Helsinki, ICH GCP guideline and Malaysian Guideline for Good Clinical Practice. Hence, a proper subject information sheet containing more information with regards to the trial is needed. In this subject information sheet, certain issues have to be addressed:

1. Who owns the results? If the results are not told to the participants, this needs to be stated clearly in the informed consent form.
2. If the echocardiogram finds abnormality, what would be the follow up procedure?
3. The participants' confidentiality (participants' identity) needs to be preserved and reassured in the form.
4. Use of posters or letters in the recruitment process needs ethical approval.
5. Elaborate more in layman term to the procedure the participants are undergoing, eg. echocardiography is a non-invasive procedure that involves….
6. Financial reimbursement plan needs to be stated clearly in the informed consent form.

In short, the 21 elements in the informed consent form and written subject information found in the Malaysian Guidelines for Good Clinical Practice, second edition 2004, should be used as a guide in formulating this informed consent form. You can get a copy of this form online at http://www.info-kinetics.com/JPEC2.html#JPEC2top .

The protocol also lacks information on selection of participants and the inclusion and exclusion criteria were not found. Generally, complying with Malaysian Guideline for Good Clinical Practice, clinical trial protocol should comprise of the below topics (whichever is applicable):

1. General information including protocol title, version number and date; the name and address of the sponsor; name and title of person authorized to sign the protocol; name and title of the investigators, their site address and contact number; just to name a few.
2. Background information including name or description of the investigational product; summary of known potential risks and benefits to human subjects; description and justification of the objective of the trial; statement stating that the trial will be conducted in compliance with the protocol, GCP and applicable regulatory requirements; description of the population to be studies and reference to relevant literature and data.
3. Trial objective and purpose
4. Trial design
5. Selection and withdrawal of subjects
6. Treatment of subjects
7. Assessment of efficacy
8. Assessment of safety
9. Statistics
10. Direct access to source data/documents
11. Quality control/assurance
12. Ethics
13. Data handling and record keeping
14. Financing and insurance
15. Publication policy

This is adapted from the Malaysian Guidelines for Good Clinical Practice, second edition 2004 under section 6.

We look forward to hear from you soon.

Thank you.

Yours sincerely,

Dr. Tan Wan Lin
Secretary

7.3.Royal Free Hospital Ethical Approval

ROYAL FREE world class

Royal Free Hampstead NHS
NHS Trust

Joint Research Office

Office Location:
R&D Office
Admin Corridor Room G649
Medical School Building

Postal Address:
Royal Free Hampstead NHS Trust
Pond Street
London NW3 2QG

Email: **hameedah.bogle-dawoud@nhs.net** Tel No. **020 7472 6163** ext: 38900 Fax No **020 7431 6209**
Web-sites:www.royalfree.nhs.uk; www.ucl.ac.uk

4th January 2012

Dr Gerry J Coghlan
Royal Free Hampstead NHS Trust
Department of Rheumatology & Cardiology
Pond Street
London
NW3 2QG

Dear Dr Coghlan

RFH R&D Ref: **8410**(Please quote in all correspondence)
REC Ref: **09/HOI01/21**
Title: **Multi Ethnic Right Ventricular Assessment in the normal heart using two dimensional and three dimensional imaging and myocardial speckle tracking**

Thank you for registering the above study with the UCL/UCLH/RF Joint Research Office (RFH Trust Site). I am pleased to inform you that your study now has local R&D approval to proceed and recruit participants at Royal Free Hampstead NHS Trust.

Please note that all documents received have been reviewed and this approval is granted on the basis of the key documents provided which are ethically approved by the Research Ethics Committee:

Document	Date
REC approval and approved documents	11/03/2009 **and** 21/05/2010

As Principal Investigator you are required to ensure that your study is conducted in accordance with the Department of Health's Research Governance Framework for Health and Social Care (2nd edition 2005) and that all members of the research team are aware of their responsibilities under the Framework.

This R&D approval is conditional upon you complying with all requirements of the Research Ethics Committee notice of favourable opinion and other any relevant regulatory bodies.

Please find attached the conditions of the R&D approval and a reminder of your responsibilities as a researcher and ensure that both yourself and the research team are familiar with and understand the roles and responsibilities both as a team and individually.

Please do not hesitate to contact a member of the team with regards to assistance and guidance for your research.

Yours sincerely

Dr Adele K. Fielding & Dr Emma C Morris
Joint Directors of Research and Development
Royal Free Hampstead NHS Trust

NB: It is essential to keep the R&D Unit informed on any updates regarding studies including patient recruitment

The Royal Free Hospital | The Royal National Throat Nose and Ear Hospital

The Royal Free Hampstead NHS Trust

7.4. Echocardiographic protocol

Operator: 3+ cycles

Study Number:

1) PLAX cycles
2) Colour AoV & MV
3) a) M-Mode through AoV/LA
3) b) M-Mode through LV
4) TV Inflow
5) TV colour
6) PV
7) PV Colour
8) PSAX Pap muscle level
9) PSAX MV Level
10) Apex
11) PSAX AoV level
12) Colour through the TV
13) Colour through the PV
14) PW in the RVOT
15) CW through then PV (PR)
16) RVOT picture (optimized)
17) Colour across the inter atrial septum
18) Colour over the AoV

Adjust Frame rate to 60 – 80

19) PSAX Pap muscle level
20) PSAX MV Level
21) Apex

Adjust frame rate back to normal.

22) Apical 4 chamber (LA & RA volume)
23) Dual Focus on LV
24) Apical 2 Chamber View
25) Apical 3 chamber View
26) Colour AoV in 3 chamber
27) TVI (frame rate >150) LV only
28) TDI PW Lateral wall (breath hold)
29) TDI PW Septum (breath hold)

Adjust Frame rate 60 – 80

30) LV Only
31) Apical 2 chamber
32) Apical 3 chamber

Normal Frame Rate

33) Apical 4 chamber

34) PW through MV (E/A ratio)

35) CW through MV (MV PHT)

36) Colour through MV

37) CW through MV (MR Jet) (reduce LOW REJECT if necessary)

38) PW LVOT

39) CW AoV

40) Colour AoV

41) Colour Interventricular septum

42) Colour Atrial Septum

43) Pulmonary Vein turn colour scale down to 40

44) PW into the pulmonary vein (systolic/diastolic flow)

45) RV- 4 chamber picture. Focus on free wall.

46) Resize to include RV & LV only

47) Colour across the TV

48) CW across TV (TR Jet Reduce low velocity jet reject)

49) PW across the TV inflow (E:A pattern)

50) TAPSE

51) Focus on RV TVI (Frame rate >150)

52) PW TVI of RV freewall

53) RV (Frame rate 60 – 80)

54) Apical 4 chamber LV Triplane

55) LV 9 slice acquisition

Stop breathing
4D
"breath in/out"
Full volume (acquires 4 cycles worth)
Image store
Cine exit
9 slice
check for stitch artefacts
9 slice exit
Image store

56) RV 9 slice as above but focus on RV with LV apex, and MV annulus in picture

57) Subcostal view

58) IVRT collapse

59) Suprasternal view

60) Colour down the arch

61) PW down the arch
62) CW down the arch